

Individual Identification, Disease Monitoring and Home Range of

Leiopelma hamiltoni

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Abstract

Amphibian populations are declining on a global scale and although disease outbreaks are a commonly accepted hypothesis they are not the only one. My aims for my thesis were to study the home range of *Leiopelma hamiltoni*, to determine whether a photographic database could be used to individual identified them and monitor the health status of the population.

Habitat loss is a possible cause. For this reason monitoring an animals' home range is a possible method to detect early impacts the population is facing. By tracking 12 *L. hamiltoni* within a 12 m x 6 m grid on Maud Island, it was shown that the home range size can vary from 0.5 m² to 25 m² based on the minimum convex polygon method.

However, to track multiple individuals it is important to be able to distinguish among frogs. The commonly used methods of identification, such as toe clipping, pose potentially detrimental effects. Therefore, non-invasive methods based on natural markings need to be established. Through the use of the dark pigmented patterns found on the skin of *L. hamiltoni* individuals can be identified on recapture with a mean accuracy of 93%.

By developing a database to maintain the photographs used for individual identification, the database can also be used to monitor the status of the population. During 2003 numerous *L. hamiltoni* were observed with denuded patches predominantly on the facial region. By monitoring five individuals within the captive facility at the University of Canterbury it was discovered that frogs appear to be able to cure themselves.

Through researching the home range requirements and developing a photographic database to monitor the population status of *L. hamiltoni*, it will aid in the management of ensuring the long-term survival of this archaic species of frog.

Preface

The overall aim of my research was to develop potential methods for aiding the management and long-term survival of *Leiopelma hamiltoni*.

In Chapter 1, I review the literature concentrating on two main topics: (1) hypotheses that may explain the decline of amphibian populations on a global scale; and (2) home ranges, and why the maintenance of a home range may be important for amphibians. In this chapter I also introduce my study animal, *Leiopelma hamiltoni*.

In Chapter 2, I address the first aim of my research, developing a photographic technique for individual identification of *L. hamiltoni*.

In Chapter 3, I consider *L. hamiltoni* home ranges. The first aim of this chapter is to determine whether this species has a home range. Once this is demonstrated, I next determine the size of individual home ranges and the extent of overlap of individuals' home ranges. Information regarding my study site, Maud Island, also is included, this being the locality of most *L. hamiltoni*.

In Chapter 4, I use photographic records as a tool for monitoring population health status. Disease appears to be the most parsimonious and most widely accepted explanation for amphibian declines. Therefore, alternative monitoring methods need to be established. The main objective of chapter was to develop a method by which photographic records could be used as a tool to monitor the health of *L. hamiltoni*, both over a short timeframe and over a much longer timeframe.

Chapter 5 is a general discussion of the results from each of my research chapters in relation to the points raised within the general introduction.

Chapter One

Introduction

1.1 Amphibian Decline

Amphibian populations are declining worldwide (Waldman & Tocher, 1998; Houlahan *et al.*, 2000) and many species are already believed to have become extinct (Lips *et al.*, 2003; Santiago *et al.*, 2003; Burrowes *et al.*, 2004). Ideas proposed to describe the causes of declining populations vary greatly. Pollutants may be responsible for the declines (Hecnar, 1995; Lips, 1998; Harris *et al.*, 2000; Hayes *et al.*, 2002; Saura-Mas *et al.*, 2002; Brown Sullivan & Spence, 2003); however, the effects of pollution vary greatly depending on the amount, duration of exposure and the development stage of the amphibian at the time of exposure. Hayes *et al.* (2002) suggested that exposure to herbicides caused gonadal abnormalities, such as retarded development and hermaphroditism. The long-term effect of this may be a reduced reproductive rate, resulting in a declining population. Pesticides could be responsible for the declining amphibian populations within the Central Valley of Sierra Nevada as dead frogs found within that area have tested positive for agricultural pesticide residue in their bodies (Davidson *et al.*, 2002).

Davidson *et al.* (2002) monitored eight populations in the Central Valley, and showed that four populations were strongly affected by wind-drifted pesticides in the area. Two were affected by urban and agricultural use of the surrounding area, but the other populations did not show positive results linking agricultural pesticides to their decline, as they may have been exposed to sublethal doses. Exposure to the pesticides such as agrochemicals as an adult may reduce immune function, making the amphibians vulnerable to diseases. Alternatively, sublethal doses of pesticides may affect the individuals' mobility reducing their ability to survive in a modified environment. Hayes *et al.* (2001) showed that herbicides affected

gonadal abnormalities, which means it is possible that the pesticide residue is affecting the reproductive success of frogs within the area. The effect need not be as dramatic as gonadal abnormality. For example, reduced fertility levels of one or both sexes might have severe effects on amphibian populations. In species such as the green frog, *Rana clamitans*, in which large clutches are produced as an apparent adaptation to overcome the high mortality rate of tadpoles, a reduction in the number of eggs laid over several breeding seasons could potentially result in a dramatic decline in population numbers.

Habitat loss (Drost & Fellers, 1996; Johnston & Frid, 2002) and environmental changes (Laurance, 1996; Lips, 1998; Pounds *et al.*, 1999; Blaustein *et al.*, 2001; Kiesecker *et al.*, 2001) are other proposed explanations for amphibian declines. Although there is evidence that potential habitat loss through forest fragmentation has not affected some amphibian populations (Chazal & Niewiarowski, 1998; Silva *et al.*, 2003), Johnston and Frid (2002) showed that amphibians are indeed affected by loss of habitat. Pacific giant salamanders, *Dicamptodon tenebrosus*, have been significantly affected by clear-cut logging. The salamanders in the clear-cut area spent more time in subterranean retreat sites and had smaller home ranges than the individuals within the forested area and the riparian buffer strips. Also, the salamanders in the clear-cut area were more dependent on precipitation than both the populations in the forested area and the riparian buffer strips. While the evidence obtained by Davidson *et al.* (2002) indicated that there was no link to UV-B and climate change and the eight amphibian populations declining in the Central Valley, California, it may be possible that climate change and UV-B are affecting the amphibians within the area but with the effect being indirect and therefore not so readily detected.

A consequence of UV-B or change in climate may be that the frogs suffer from high stress levels, with stress causing impaired immune response (Davidson *et al.* 2002). This might have resulted in four populations observed by Davidson (2002) declining as indirect

effects of pesticides. The combination of the pesticide exposure and a weakened immune response may have resulted in the frogs' inability to fight infection from opportunistic pathogens. Environmental changes may not have a direct effect on amphibian populations; however, it is possible that changes in environmental conditions may affect the parasite populations that rely on amphibian hosts for one of their life stages (Kiesecker, 2001). There are many examples where parasites affect amphibian populations, varying from causing malformation which directly affect survivorship (Johnston *et al.*, 2002) to decreasing the individuals' ability to select oviposition sites (Kiesecker & Skelly, 2000) or compete for food and territories (Maksimowich & Mathis, 2000). Therefore a heavily parasitised population may result in weakened immune systems as the individual's immune system may be concentrated on reducing the effect of the parasites. increasing the risk of opportunistic pathogens. Alternatively future offspring may not have the strongest genetic fitness due to poor mate selection which can decrease the long-term survival of a population.

Laurance (1996) proposed that unusual weather patterns (reduced rainfall and extended period of drought) may be possibly responsible for Australian frog population declines. It is possible that a higher desiccation rate might be the proximate cause of death or the extra stress experienced by the frogs during extended drought periods may increase their susceptibility to opportunistic pathogens. The question of importance here is whether these declines are similar to other years with similar weather patterns, and are therefore a natural phenomenon that has not been observed frequently enough for a pattern to have been recorded. However, if these declines are higher than previously recorded or if mortality rates of the affected populations are at a level that the long-term survival of the populations is in doubt, then weather change is a plausible explanation for the declines.

In recent years the Australian rainforest frog populations have suffered catastrophic declines. Efforts to explain global declines of amphibian populations often lead to discussion

of outbreaks of infectious diseases (Crawshaw, 1997; Daszak *et al.*, 1999; Bosch *et al.*, 2001; Muths *et al.*, 2003). Laurance *et al.* (1996) proposed that the declines occurring in Australia are the results of an exotic pathogen that may have been introduced by increased human activity, especially in the aquarium fish trade. However, little evidence of disease has been documented, casting doubt on this explanation (Alford & Richards, 1997; Hero & Gillespie, 1997). Alford and Richards (1997) indicated that Laurance *et al.*'s (1996) hypothesis regarding an exotic pathogen causing the amphibian declines is not plausible because the pathogen would have to travel over vast areas of arid land and spread at the rate of 100 kilometres per year. While Alford and Richard did not rule out the disease hypothesis, they suggested that more conclusive data are needed. One possibility that needs to be considered is that the disease was moved over the arid areas by human activity within and between infected and non-infected areas.

Despite the conflicting evidence, disease is still a commonly accepted explanation for amphibian declines and there are many examples where a plausible link has been identified between population decline and infectious disease outbreak. In particular, many diseases are known to infect amphibians' skin and because the skin is vital for water absorption and aiding respiration (Pessier, 2002) diseases affecting the skin of an amphibian can have fatal consequences. Red leg is a commonly known skin disease with the bacteria responsible for the disease commonly found in most environments. The major symptom of a red leg infection is that the ventral surface of the amphibian's body, especially rear legs and abdomen region, takes on a red colour (Pessier, 2002). The animal also can be lethargic. Many different bacterial infections, (*Aeromonas hydrophila*, *Pseudomonas* spp, a wide variety of gram-negative species and occasionally gram-positive species) are known to cause red leg symptoms.

However, the difficulty attributing declines to many bacterial skin diseases is that many bacteria species are commonly found in the normal environment. *A. hydrophila* and *Pseudomonas* spp. are examples of bacteria that can be found in the environment but also can become fatal for some frog species (Pessier, 2002). It may be possible that many of these bacterial infections only become dangerous to the individuals as secondary infections, after an injury or the animal has been exposed to extreme stress (due to environmental or physical conditions). Traumatic and environmental skin diseases can be as simple as an abrasion from handling. Alternative it might result from scraping on substrate during dry condition. Traumatic skin diseases sometimes result from bites from conspecifics', predators or prey. Deeper wounds can be the result of fighting between males during breeding season or territorial defence. These wounds can then become infected with secondary bacterial or fungal diseases (Pessier, 2002).

An amphibian with an open skin wound might become vulnerable to *Mycobacteriosis* which is one of these opportunistic pathogens (Pessier, 2002). However, chytridiomycosis is the disease that has been most strongly implicated in the declines of many frog species throughout the world. There are pockets of species that are not been effect while other species with the same area have completely disappeared (Carey, 1993). Chytrid fungus affects keratinised skin, and as the mouthparts of tadpoles are the only keratinised part, the tadpoles are not directly affected by the disease but act as carriers of the disease until metamorphosis (Daszak *et al.*, 1999; Waldman *et al.*, 2001).

In recent years, a population of boreal toads, *Bufo boreas*, in Colorado has show dramatic declines (Muths *et al.*, 2003). Mark-and-recapture studies (Muths *et al.*, 2003) have shown this toad population declining from 78% of marked frogs surviving from 1991 to 1994 to 45% marked frogs being recaptured in 1995. By 1998 and 1999, the survival of the marked frogs was down to only 3%. Histology showed the characteristic features of chytrid on six

wild toads. As only six individuals were tested, perhaps chytrids are not the main cause of the decline, but there is a secondary disease. However, Bosch *et al.* (2001) studied a larger sample size, 700 dead post-metamorphic toads from an estimated population of 5000 tadpoles collected in early 1999, and an additional 50 dead post-metamorphic toad after the 1999 breeding season, only ten tadpoles were observed. Scanning electron microscopy and histological techniques showed chytrid fungus was present on the skin of the dead toads. Bosch *et al.* (2001) analysed water quality at the study sites in an effort to eliminate the possibility of a change in water quality as the cause of the sudden declines. As water chemistry tests were conducted on the pond, to ensure the declines were not a side effect due to change in the water chemistry it increased the conclusiveness that chytrid fungus was the cause of the declines.

However, even with all the evidence for particular causes for the different population declines, no definite explanation has emerged for declining populations on a global scale. Various populations that are declining continue to contradict current explanation. The ability of amphibian species to adapt complicates efforts to explain population declines. Many diseases that affect amphibians are associated with the skin and, the skin of amphibian species is the first line of defence against diseases. Rollins-Smith *et al.* (2002) showed that amphibians have antimicrobial peptides that are effective against chytrid fungus, *Batrachochytrium dendrobatidis*, and *Basidiobolus ranarum*. *B. ranarum* was found in the declining populations of Wyoming toads. However, these peptides were not effective against *Aeromonas hydrophila*, which is an opportunistic pathogen commonly found in the environment. Other defence mechanisms against diseases have been documented. The tadpoles of the red-eye tree frog (*Agalychnis callidryas*) are able to hatch early if the egg mass becomes highly infected with a filamentous ascomycete (Dothideales: Phaeosphaeriaceae) (Warkentin *et al.*, 2001).

Early hatching occurred only after the tadpoles have reached a development stage at which their survival in the environment is possible. How the early hatching is induced is unknown; but one potential method is for the tadpoles to sense changes in the chemical surroundings in the egg mass. This ability might be disrupted by toxins produced by the fungus (Warkentin *et al.*, 2001). There are trade-offs with this defence mechanism. The tadpoles that hatch early are not as well developed as those that remain in the egg mass for the whole time and therefore may not be able to cope as well in the environment. However, this defence results in some of the tadpoles surviving to adulthood, compared to the one hundred percent mortality they would have experienced had they remained in the egg mass.

Confirming that chytrid fungus is present within a population involves obtaining a skin sample, either sloughed skin, skin scraping or by removing an individual's digit. Skin scraping and digit removal can be very stressful to the individual amphibian and may increase the individual's susceptibility to illness. The effect of stress levels may be especially significant in research on *Leiopelma* species, as these frogs seem to become stressed very easily.

Having found that there have been many populations where both dramatic declines have occurred and chytrid tests have proven positive there is the danger that chytrid could potentially become the main focus of research; with other plausible causes being overlooked until too late.

There are many viruses that are potentially lethal to amphibian species. They may be directly lethal or they may be indirectly lethal through decreasing the effectiveness of the animal's immune system (Crawshaw, 1997). Perhaps during time of extreme stress (e.g. during times of drought or prolonged handling) functioning of the amphibian's immune system is impaired, making individuals more vulnerable to infectious diseases.

With the current decline of many amphibian populations, it is important to establish new, alternative methods for monitoring these declines. Some of the declines that have been

attributed to disease may in fact be a consequence of a loss in home range. Johnston and Frid (2002) showed that Pacific giant salamanders, *Dicamptodon tenebrosus*, are significantly affected by clear-cut logging. Salamanders in the clear-cut area spent more time in subterranean retreat sites and had smaller home ranges. In addition, the salamanders in the clear-cut area were dependent on precipitation to a far greater extent than the population in the forested area and the population in the riparian buffer strips.

This may be the result of human impacts or it may indicate that individuals cannot obtain and maintain sustainable home ranges due to a reduced fitness or resource availability. Research on home ranges for many amphibian populations are needed, as some of the current declines may be a result of the either increased competition for home ranges or decreases in the size of home ranges brought about by competition. This will result in reduced availability of resources needed for survival or attracting a mate (Kleeberger, 1985). Current population estimates based on mark-and-recapture studies may not be sufficient to detect changes in population dynamics and habitat usage. For this reason, it may be important to conduct population studies on a fine scale and monitor home ranges as a way of determining potential changes in population dynamics. It is difficult to study the home range of many amphibian species because of the habitat in which the amphibian lives or the amphibian's life history traits. However, *Leiopelma hamiltoni* seems to be ideally suited for home range studies due to its behavioural traits and the habitat in which it is found. This frog is restricted to three small offshore islands, with the population on the largest island, Maud Island, being estimated at 20,000 individuals within a 16-hectare forested area.

1.2 Home Range

Over the years the definition of home range has changed and now there is some confusion as to whether territoriality and homing behaviour provide the best indication an animal's home range.

Brown (1975) described an animal's home range simply as the area in which the animal normally lives and fulfils daily requirements. The home range of an animal does not include any migratory area, such as communal breeding sites. The area covered during emigration and dispersal events cannot be included in the area defined as its home range as these behavioural events are not classified as everyday activities. The presence or absence of behaviours such as territoriality should not be used as an indication of an animal's home range. In other words an animal may have a home range but not actively defend some or all of it. Some animals with home ranges will only actively defend various areas, such as retreat sites (Wells, 1977).

Within an animal's home range, there may be a site where the animal spends a greater proportion of time during the day or night (Brown, 1975). These areas within the home range area where the animals spend more time may be energy-efficient foraging sites (Test, 1954; Kleeberger & Werner, 1982; Gabor & Jaeger, 1995) or alternatively they may be calling or breeding sites (Kleeberger & Werner, 1982; Heying, 2001) or a retreat site (Test, 1954; Kleeberger & Werner, 1982; Kleeberger, 1985; Watson *et al.*, 2003). If the animal is territorial it may display this behaviour at these particular sites (Wells, 1977).

There are many factors that may influence the size of a home range. For example, the sex of the animal may influence the size of its home range. For many species the male can have a large home range that may overlap several females' territories (Ferner, 1972; Mathis, 1991; Bonaccorso *et al.*, 2002; Hingrat *et al.*, 2004). Alternatively, the quality and quantity of resources in the area may affect the size of the home range. The size of the home range also can be influenced by the size of the animal; however, because using body size is not always

appropriate or reliable caution is needed. Kleeberger and Werner (1982) showed that size is not always suitable for determining home range, as the smaller individuals of *Plethodon cinereus* generally had the larger home range.

Although the literature on home ranges is very extensive (Ferner, 1972; Semlitsch, 1981; Kleeberger & Werner, 1982; Broomhall *et al.*, 2003; Kalmmler *et al.*, 2003; Monsnire *et al.*, 2003; Muths, 2003; Schmidt *et al.*, 2003; Taylor & Skinner, 2003), there has been little research on home ranges of amphibian species, with most of what we know for amphibians being on salamanders. As the global phenomenon of amphibian declines continues, many conservation groups and organizations are actively working to protect the species within biodiversity hotspots. However, it is not always enough just to protect the individual species, for many species have special requirements and, in these cases, it is important to protect the habitat and ecosystem as well. For this, it is imperative to know how large an area is needed, and if the species can adapt to a modified environment. One important aspect of an animal's habitat requirement is the size of the home range and the resources within the home range that are needed to make the area suitable for the individual's needs. Potentially important resources include food (Test, 1954; Townsend & Jaeger, 1998), breeding sites (Wiewandt, 1969) or potential mates (Wells, 1977; Gabor & Jaeger, 1995), and retreat sites (Sexton, 1960; Jaeger *et al.*, 1986; Lang & Jaeger, 2000; Gauter & Miaud, 2003).

An animal with a home range may or may not actively defend their area. A species that actively defends their home range or breeding site is said to be territorial. Many amphibians may share a larger home range while defending a calling or breeding territory (Wiewandt, 1969).

For many amphibians species there is an increase in territorial behaviour during the breeding season; however, some species only show territorial behaviour during the breeding season. In the case of the male green frogs, *Rana clamitans*, male aggression was only

observed during the breeding season (Shepard, 2004). This suggests that the major limiting resources are females (Wells, 1977) and breeding sites. This means that during the breeding season there is a greater need to acquire and protect these resources and during the remaining part of the year the resources individuals require (e.g. food and retreat sites) are plentiful; this might result in competition for these resources becoming unnecessary. Aggression by the male green frogs, *Rana clamitans*, during breeding also may be a strategy favoured by sexual selection, with the females mating with the strongest and fittest males. There are other amphibian species that are not territorial and it is not uncommon to find several individuals sharing the same retreat or breeding site. *Leiopelma hamiltoni* is one of the species that seems to be a non-aggressive species (i.e. it does not remove or deter invading frogs). Although, there is insufficient known about the behaviour of this species to say conclusively that they are a non-aggressive species. It is not uncommon to find several individuals of *L. hamiltoni* under the same retreat site (personal observation).

For an individual, the benefits obtained by defending a territory, which may be a breeding or retreat site, need to be greater than the costs involved in defending it. Important costs might include reduction in foraging time; however, the resources within the territory (e.g. food) can determine the quality of a territory. This would mean that although an individual in a high quality territory would need to spend more time defending, the greater food resources in the territory might allow foraging time to be more energy efficient. Jaeger *et al.* (1995) showed that the food resources available within a territory are used as a method of attracting gravid females. The presence of termites in a male's faeces was far more attractive to females than the presence of ants. Termites are rare within the salamanders' territories, but are far more energy efficient as they contain less keratin and are easier to digest. Therefore, a salamander defending an area that has a termite nest in it has increased advantages in terms of mating success, as well as the advantage of having high-quality food. It is not only gravid

females that some species of territorial salamander will tolerate in their territory. During stressful foraging times, juvenile salamanders entered the territory of adult salamanders (Jaeger, 1995). Juvenile salamanders usually forage within the leaf litter. However, as the habitat dries, foraging becomes more difficult, and the juveniles move into the adults' areas, which remain damper longer due to the presence of cover objects. Jaeger showed that there is still territorial defence by the adults if the juvenile was unfamiliar to the resident male. This behaviour pattern is consistent with the "dear enemy" hypothesis (Fisher, 1954), as the juveniles do not act as territorial neighbours.

There are also the physical costs involved, including the possibility of direct injury due to fighting. One method of fighting amongst salamanders is to bite the opposition (Cupp 1980; Keen & Sharp, 1984; Jaeger & Forrester, 1993). These bites become a potential site for infection or, if a severe injury occurs, the victim may become unable to forage for food.

There are many ways in which an animal can mark and identify its own territory and identify that of both known and unknown neighbours. Many species use faecal pellets as markers (McGavin, 1978; Simon & Madison, 1984; Jaeger *et al.*, 1986; Anthony, 1993). The usage of faecal markers is not only more energy efficient compared to the actively defending one's territory, but also potentially can provide information about the territory-holder's sex (Jaeger, 1993) and relative fitness (Simon *et al.*, 1997).

Although males' territories may overlap or include multiply female's territories (Ferner, 1972; Mathis, 1991; Bonaccorso *et al.*, 2002; Hingrat *et al.*, 2004), it is not only the male that can show territorial behaviour. Female red-backed salamanders (*P. cinereus*) not only can recognise faecal pellets of other females but also physically squash them and they sometimes return from their burrow to re-examine pellets. Perhaps territories with high quality food resources and with possibly one or more high quality males in close proximity

are very limited. Therefore females may, compared to male, be forced to become more aggressive, regardless of the season.

This behaviour is not limited to the salamanders. *Leiopelma hamiltoni*, an archaic species of frogs restricted to offshore islands of New Zealand are not physical able to vocalise (Newman, 1982; Bell *et al.*, 1984; Bell, 1995; Crook, 1997). It relies instead on chemosignals. Waldman and Bishop (2003) found that the preference shown by *L. hamiltoni* for the paper towel that they had marked themselves increased the further away the experimental frog was collected from the test frog. It is possible that this species of frog does show territorial behaviour when the conspecific is unfamiliar and possibly, the distance between individual frogs may be an indication of relatedness. An alternative explanation is the “dear enemy” hypothesis (Fisher, 1954).

Although many modern frog species may also communicate chemically, the main method recorded and observed for most is by vocalisation. For many species, vocalisation is used not only to maintain territories (Emlen, 1968; Wiewandt, 1969) but also as a method of attracting potential mates (Lutz, 1960; Fellers, 1979).

1.3 Study Animal

There are three extinct species and four extant species (Bell, 1978; Bell, 1982; Bell *et al.*, 1984; Bell, 1994; Bell, 1995). Of the extant species *L. hochstetteri* is a semi aquatic species, with the other three species being entirely terrestrial (Bell, 1982; Bell *et al.*, 1984). Recently, the Department of Conservation reclassified the population of *Leiopelma* on Stephen’s Island as *Leiopelma hamiltoni* making it a separate population from the population on Maud Island (Bell *et al.*, 1998) and the recent translocated Motuara Island population (Crook, 1997). The Maud Island and Motuara Island populations were renamed as *Leiopelma pakeka* (Bell *et al.*, 1998). However, a more recent study (Holyoake *et al.*, 2001) used a

different technique and the results indicated that there was not sufficient genetic separation for the different populations to be classified as different species. Therefore, for the purpose of this research the Maud Island population will be referred to as *Leiopelma hamiltoni*.

Leiopelma species do not have a free-living tadpole stage (Newman, 1982; Bell *et al.*, 1984; Bell, 1995; Crook, 1997); all the development of the offspring is within a jelly-like substance which surrounds the developing offspring. The offspring emerge at the froglet stage, where the male carries the froglets on his back (Bell, 1982; Bell, 1984; Bell, 1995) until their tail is fully absorbed and they are able to fend for themselves. The male broods the eggs prior to hatching, meaning the male stands over the eggs. The reason behind this is unknown. This information is based on observations of captive *L. hamiltoni* (Bell 1984) and observations of *L. archeyi* (Bell, 1984) as this behaviour has not been observed in the wild populations of *L. hamiltoni*.

Adults do not vocalise except with high-pitched squeak alarm calls (Bell, 1982; Newman, 1982; Bell *et al.*, 1984; Crook, 1997; Eggers, 1998). The individuals make these calls only when they are highly stressed. *Leiopelma* lacks the vocal sac and external tympana found in modern frogs. *Leiopelma* species have retained the presence of free ribs, compared to the fused ribs found in modern frogs, as well the adult frogs retain their tail-wagging muscles even though they do not possess a tail (Bell, 1978; Bell, 1982; Bell, 1984; Bell *et al.*, 1984; Bell, 1995; Crook, 1997; Eggers, 1998).

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Chapter Two

Photographs As A Tool For Individual Identification

2.1 Introduction

With the global decline of amphibians (Crump *et al.*, 1992; Richards *et al.*, 1993; Lips, 1998; Daszak *et al.*, 1999; Houlahan *et al.*, 2000; Kiesecker *et al.*, 2001; Muths *et al.*, 2003) new techniques are being employed to enhance the knowledge of many species without exposing individuals to an increased vulnerability to pathogens or reduction in survival rate. Individual identification based upon natural markings is one method. The idea of individual identification is not a new concept and has been used for many years. The theory behind individual identification involves the use of physical markings, patterns or colouration to distinguish between conspecifics. The characteristics used for identification need to be permanent once the amphibian has obtained the adult form and they need to be distinctive enough to avoid confusion between individuals. The main reason behind the use of natural marking for individual identification is that it enables identification to occur without the need to handle the animal for extended periods.

Individual identification based on physical markings or colouration is commonly used for many different species (Hurst, 1988; Rugh *et al.*, 1992; Bretagnolle *et al.*, 1994; Doody, 1995; Meyer & Grosse, 1997; Davis & Ovaska, 2001; Neumann *et al.*, 2002). The use of colouration on the dorsal fin of the short-beaked common dolphin, *Delphinus delphis*, (Neumann *et al.*, 2002) was used as an identification methodology as this species of dolphin compared to bottlenose dolphins, does not have as many nicks on the fin, which is the more commonly used method for

dolphin identification.

However, this is not a commonly used method for amphibians. There are a few species of salamanders in which natural markings are used to identify various individuals. Marking amphibians with waistbands (Donnelly *et al.*, 1994), PIT tags (Donnelly *et al.*, 1994; Perret & Joly, 2002), radio transmitters, or toe clips (Davis & Ovaska, 2001) are all common methods to enable several individuals to be distinguished between each other. The use of waistbands may introduce potentially lethal pathogens through the open wounds that can develop as a result (Doody, 1995). Potentially, waistband and radio transmitters could increase the individual's risk of predation, as parts of the devices may be able to catch on vegetation, or affect the individual's camouflage. Also both PIT tags and radio transmitters are expensive to use (Doody, 1995). The most common method of marking amphibians is by toe clipping. However, toe clipping can be detrimental to the individual's wellbeing (Doody, 1995; Davis & Ovaska, 2001; McCarthy and Parris, 2004).

Photograph identification is used for individual recognition for some salamander species. Doody (1995) used individual identification for *Ambystoma opacum* based on the appearance and number of bars on the dorsum. The technique used by Loafman (1991) relied on the number of spots on the salamander, *Ambystoma maculatum*, in different locations on the body. When this technique was tested it was shown to have a 97% accuracy rate for identifying 174 individual salamanders. Loafman's technique has the added advantage that once the number and location of the spots are recorded, it eliminates the need for repeat photographs or drawings to confirm identification.

Natural markings have not only has been used for individual identification for salamanders, but this technique can be modified and developed for other amphibian species. Meyer and Grosse (1997) showed that the technique of individual identification could be used on *Bufo calamita*. The method was based on the natural spot pattern of the ventral surface of the toads. However, the patterns did not become stable until after the first hibernation. Meyer and Grosse (1997) also showed that the patterns remained stable for over five years, which meant that this technique is suitable for long-term population studies.

The advantage of using a photographic mark and recapture technique is not only the decrease in cost (Doody, 1995) compared to other methods, but it is more reliable due to the fact that many amphibian species can lose toe digits as a result of fighting or predation (Doody, 1995; Gray *et al.*, 2002). There is also the possibility of clipped digits regenerating in salamanders over time. Davis and Ovaska (2001) showed that the rate of regeneration of clipped toes of *Plethodon vehiculum* steadily increased 35 weeks after initially being removed. In a field experiment Davis and Ovaska (2001) showed that the number of recaptured individuals that had been toe clipped was only 40% compared to 60% recapture success for fluorescent-tagged individuals. In a second season the toe clipped salamanders had gained less weight in relation to the initial body size when compared to the fluorescent-tagged salamanders, suggesting that the process and effect of toe clipping may prevent individual's optimal foraging ability. McCarthy and Parris (2004) showed through the use of Bayesian statistics that the number of digits removed from frogs and toads may influence the return rate, with the return rate decreasing with the increase in number of digits removed.

The concept of individual identification based on markings or colouration is not practicable or possible on some species of amphibians due to habitat or behavioural adaptations.

However, the *Leiopelma* genus found in New Zealand is ideally suited to individual identification. The small snout vent length of 49 mm maximum (Bell, 1982) for the largest of the three different species means that most alternative identification, such as PIT tags and radio tracking devices, are too large and the potential increase risk of pathogens makes toe clipping a potentially dangerous method of identification. Until recently toe clipping was the only method available for many of the *Leiopelma* species. Bradfield (2004) developed a technique for individual identification for *L. archeyi* based on the whether various markings on the head, face and sides of the frogs are continuous or discontinuous.

Two of the *Leiopelma* species are entirely terrestrial, while the third is semi-aquatic living in splash zones of streams (Bell, 1982), and their natural defensive behaviour of freezing when threatened, means that it is very easy to record and observe markings. The use of individual identification is vitally important for captive breeding. To date there is no way of distinguishing the sexes based on physical differences for two of the *Leiopelma* species. Macfie (2002) showed that through ultrasound of individuals, it is possible to distinguish gravid females of *L. archeyi* and *L. hochstetteri*. This technique is not only expensive and time consuming; it was not always reliable as it was difficult to clearly identify some females (Macfie, 2002). Therefore, through observing the behaviour, especially mating behaviour, of known individuals, it is possible that a morphological difference or methodology for determining each sex will be discovered. The use of individual identification is essential for captive breeding, to enable individual's health records to be maintained, and to develop a studbook to ensure genetic diversity of the captive population is maintained.

To use colouration or natural markings for individual identification, it is important to establish whether these markings change depending on the age or development of the particular

individual. Examination of photographs taken between 1976 and 1983 by Don Newman (personal communication) permits an assessment of whether the markings of *Leiopelma hamiltoni* change as the individual grows and ages. Individual identification for *L. hamiltoni* was first used by Newman (1982) as a method of obtaining a population estimate of the frogs on Stephen's Island. Newman used the markings around the lip region and estimated the population to be approximately 200 individuals. The Stephen's Island population is slightly lighter in colouration and smaller than the Maud Island population (Bell *et al.*, 1998). The majority of the population of *L. hamiltoni* is on Maud Island. Thus, for individual identification to be feasible to use on Maud Island frogs, more markings than those on the lip region are needed.

2.2 Method

Photographs were taken of 34 different individuals of *L. hamiltoni* collected during 2003 and 2004 and housed in the captive facilities at Canterbury University in Christchurch, New Zealand. The photographs were taken using a Nikon Coolpix 4500 digital camera. The flash was used to reduce the capturing of shadows. The macro mode function was used for all the photographs. Photographs were taken of the face, dorsal view, rump, ventral view and both lateral views. The camera was positioned 30 cm away from the frog and was on the same level as the frog for all the photographs except for the dorsal view when the camera was directly above the frog. For analysis, the rump and ventral view were eliminated, as the lateral views, dorsal view and the face provided clearer views.

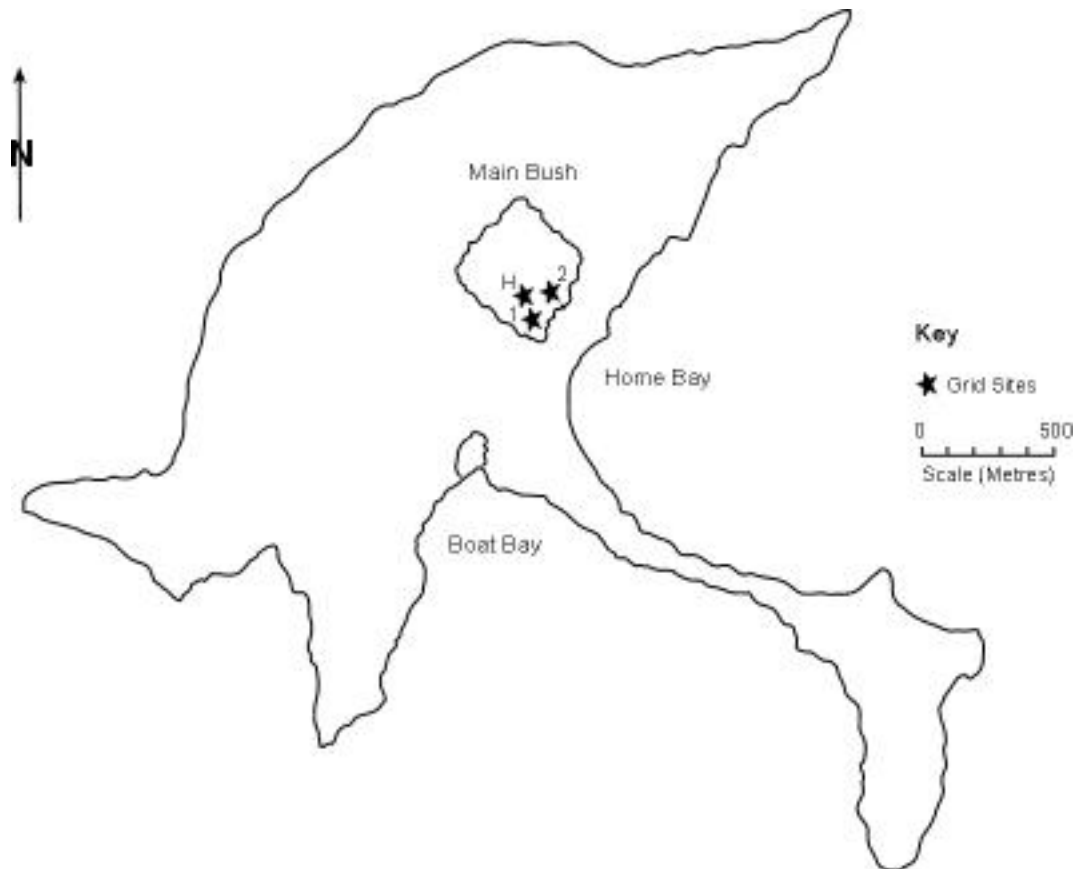


Figure 1 Outline map of Maud Island, showing grid sites in the main forest area. Site Grid 1 and Grid 2 were the historical grid sites where Don Newman photographed the frogs between 1976 and 1983, while grid site H was the location of my home range grid site (Map modified from Bell, 1995).

The first stage was to ascertain whether the markings on the frog changed with either age or the growth of the frog. Past photographs of *L. hamiltoni* taken by Don Newman at two different locations, Grid 1 and Grid 2 on Maud Island, Malborough Sounds (Figure 1) during the years between 1976 and 1983 were used to monitor whether the darker pigmented areas on the frog's skin changed over time, either in size or location on the skin's surface. Selected frogs that had repeated photographs spanning three or more years allowed for the greatest timeframe over which variation in the patterning of the frogs may occur. Three different body marks were used

for each different frog; the body marks were dark pigmented patches on the frog's skin. The main areas used for obtaining body marks were the facial markings and the lines directly posterior to the eyes on both lateral views as these marks remained clearly visible in all photographs. To identify body marks two different measurements, the length and width of each mark, were recorded using a ruler and a ratio between these measurements was obtained. Results for the length and width of the mark posterior to the left eye are in Table 1. The ratio was used to compare between the years, allowing for the growth of the frogs as well as any differences due to different camera angles and proximity of the camera to the frog.

The manually recorded ratios were then compared to measurements obtained by the computer program ImageJ v1.30 (Table 1). ImageJ is a public domain Java image-processing program, inspired by NIH Image for Macintosh computers. Analysis was conducted using an Apple Macintosh computer. Photographs were scanned in at a resolution of 150 d.p.i and then the same body marks were measured.

Table 1 Body mark ratio for the left lateral line mark of two *L. hamiltoni* individuals, EJ9 and EJ52, comparing the manually recorded values to those obtained using the computer program ImageJ. The final column in the table is the ratios obtain after using the snout-vent length (SVL) to calibrate the computer program scale.

Frog	Year	Manual length (mm)	Manual width (mm)	Ratio	Computer length (mm)	Computer width (mm)	Computer ratio
EJ9	1977	13	3	4.30	6.97	2.22	3.14
	1978	13	3	4.30	6.27	2.28	2.75
	1979	13	3	4.30	7.21	2.85	2.58
	1980	13	3	4.30	6.95	2.55	2.73
EJ52	1978	8	3	2.67	5.84	3.15	1.85
	1979	8	3	2.67	5.53	2.44	2.27
	1980	9	4	2.25	4.85	2.02	2.40
	1981	7	3	2.33	5.16	2.15	2.39

To ensure that variation between the sizes of the body marks in the different lateral views was due to natural causes and not a side effect of differing camera angles, a ratio for both sides was obtained (Table 2). Measuring both the lateral views with a clear plastic ruler over the photograph from the vent to the tip of the snout gave the SVL of the frog in the photograph. This number was then divided by the known SVL, giving a ratio (Table 2).

Table 2 Examples of the ratios obtained for the hard copy photographs taken by Don Newman between 1976 and 1983, by dividing the actual SVL with that of the SVL measured from the photographs.

Frog	Actual SVL (mm)	Measured Left SVL (mm)	Ratio	Measured Right SVL (mm)	Ratio
EJ 5	37.30	70	0.53	74	0.53
EJ 9	43.40	79	0.55	77	0.55
EJ 32	37.00	64	0.58	67	0.58
EJ 20	37.55	71	0.53	70	0.53
EJ 52	42.60	77	0.55	76	0.55
EJ63	39.00	71	0.55	72	0.55

The SVL values of the photographs taken between 1976 and 1983 were measured dorsally. To confirm the accuracy of the measurements obtained by using the computer program ImageJ, photographs were taken of the lateral views of the frogs currently held in the captive facilities at Canterbury University. These frogs had graph paper in the same plane as the frog, to enable a measurement to be obtained. Each of the grids of the graph paper measured 1 mm². There was also a ruler placed within the frame of the photograph. Each of the frogs was measured dorsally and the measurement was then compared to each of the methods of measuring the SVL of the frogs. The first method involved using the measurements on the ruler to calibrate the scale of the computer program and then measuring the SVL of the frog by drawing a straight line from the vent of the frog to the tip of the snout.

The second method counted the number of whole squares on the graph paper starting at the vent of the frogs and finishing at the tip of the snout, and the third method of measuring the

frogs involved drawing a straight line from the vent of the frog to the tip of the snout of the frog, in the same manner as the first method. The scale for this line was calibrated by entering the known SVL.

To confirm the accuracy of this measurement a line was then drawn from the beginning of the first square on the graph paper to the end of the last square and the measurement was compared to all the other SVL measurements obtained (Table 3). The manual body mark ratios were compared to those obtained by the computer program ImageJ.

Table 3 Comparison of the different measuring methods used to confirm the accuracy of the computer program ImageJ. The program was used to obtain the SVL of the frogs' sampled between 1976 and 1983 and 2003 and 2004.

Frog	Manual dorsal SVL (mm)	Manual Calibrated SVL (mm)	Ruler Calibrated SVL (mm)	# of squares	Graph Paper Calibrated SVL (mm)
Frog 1	45	49	41	50	48
Frog 2	44	46	39	48	42
Frog 3	43	47	39	46	42
Frog 4	46	51	42	48	45
Frog 5	44	49	41	48	46

Once it was ascertained that there were body marks on the frogs that remained constant and reliable over an extended period of time, the methodology for developing an individual identification based on natural marks was divided into two parts.

The first aspect involved the development of the sub-groups into which the frogs were placed in. This required a three-stage process. Stage 1 used the facial view of the frogs. The body marks that were used were the lines on the face anterior to the eyes, and the darker pigmented marks between the nostrils on the snout region. Three criteria were established to form three different groups:

- 1) Group 1 – The lines on the face anterior to the eye completely, all the way to the nostril region and joined together with nostril region. The outline of the darker pigmented marks form either a U or V shape. (Figure 2A)
- 2) Group 2 - The line on the face anterior to the eye does not join completely with the darker pigmented marks on the nostril region. Markings do not form a complete U or V shape (Figure 2B)
- 3) Group 3 – Lines on the facial region anterior to the eye remain separate (Figure 2C)

Stage 2 used the darker pigmented body marks on the animals' left lateral line. The body mark started directly posterior to the eye. Three criteria were used to separate the different lateral line patterns:

- 1) Code A - The lateral line was complete all the way to just posterior to the frog's front leg. (Figure 3A). For example frogs from Group 1 would be coded Group 1A.
- 2) Code B - The lateral line was broken before reaching the posterior side the frog's front leg, but the darker pigment continued further down the lateral line of the frog (Figure 3B). For example frogs from Group 1 would be coded Group 1B.
- 3) Code C - The lateral line was incomplete, meaning the line did not reach the posterior side of the frog's front leg and did not carry on (Figure 3C). For example frogs from Group 1 would be Group 1C.

The three code criteria developed in stage 2 were applied to all the frogs in each of the three groups established during stage 1 classification.

Stage 3 was developed to further categorise the frogs into the different groups. Stage 3 used the same body mark as stage 2, except it was on the animal's right lateral line. Three criteria were established to separate stage 3 markings:

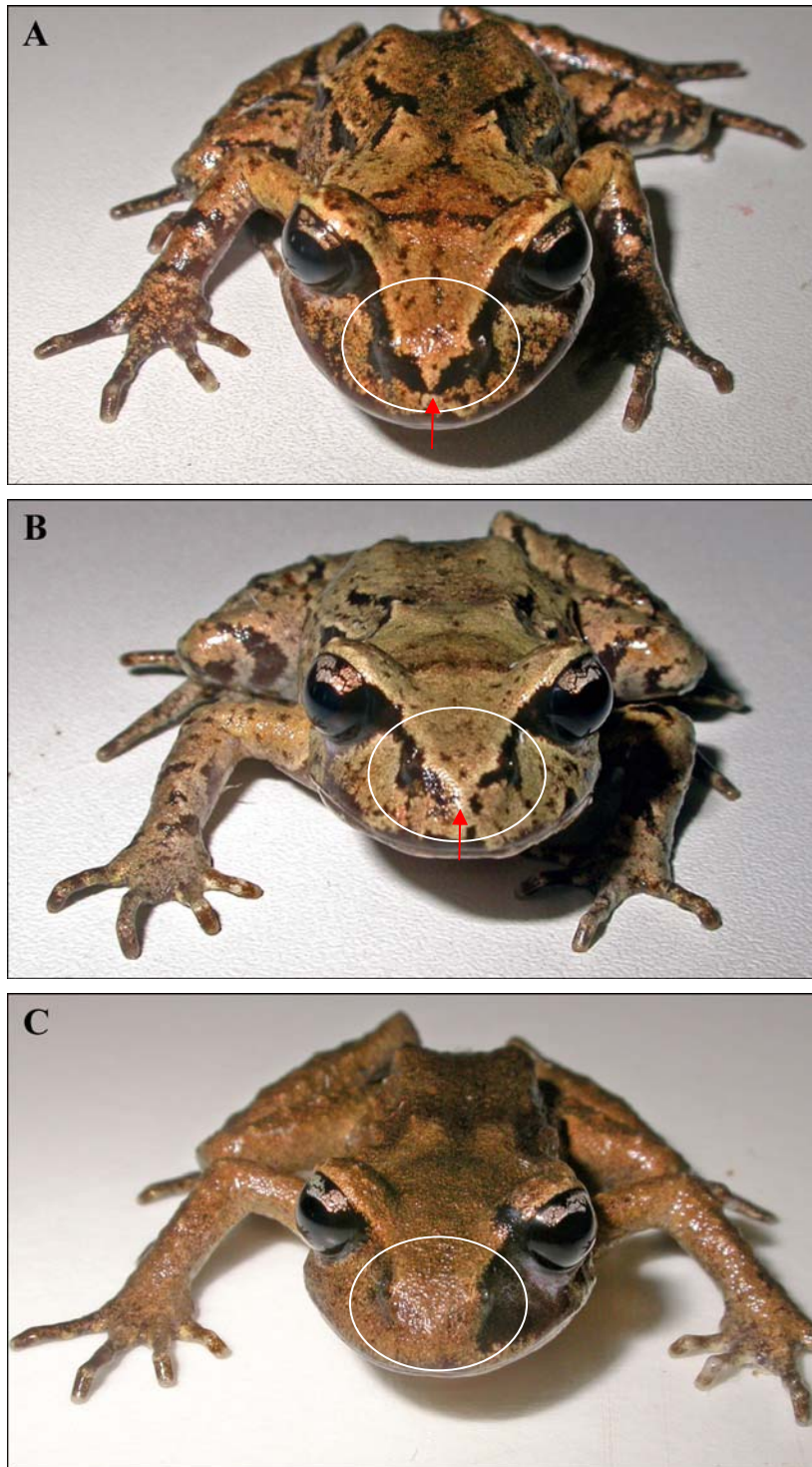


Figure 2 Facial view of head/snout markings of captive *Leiopelma hamiltoni* categorised as Stage 1. A) Group 1 – facial line markings join across front of nostril region of snout (arrowed); B) Group 2 - Markings do not connect at front of snout (arrow); C) Group 3 – Facial lines remain separate.

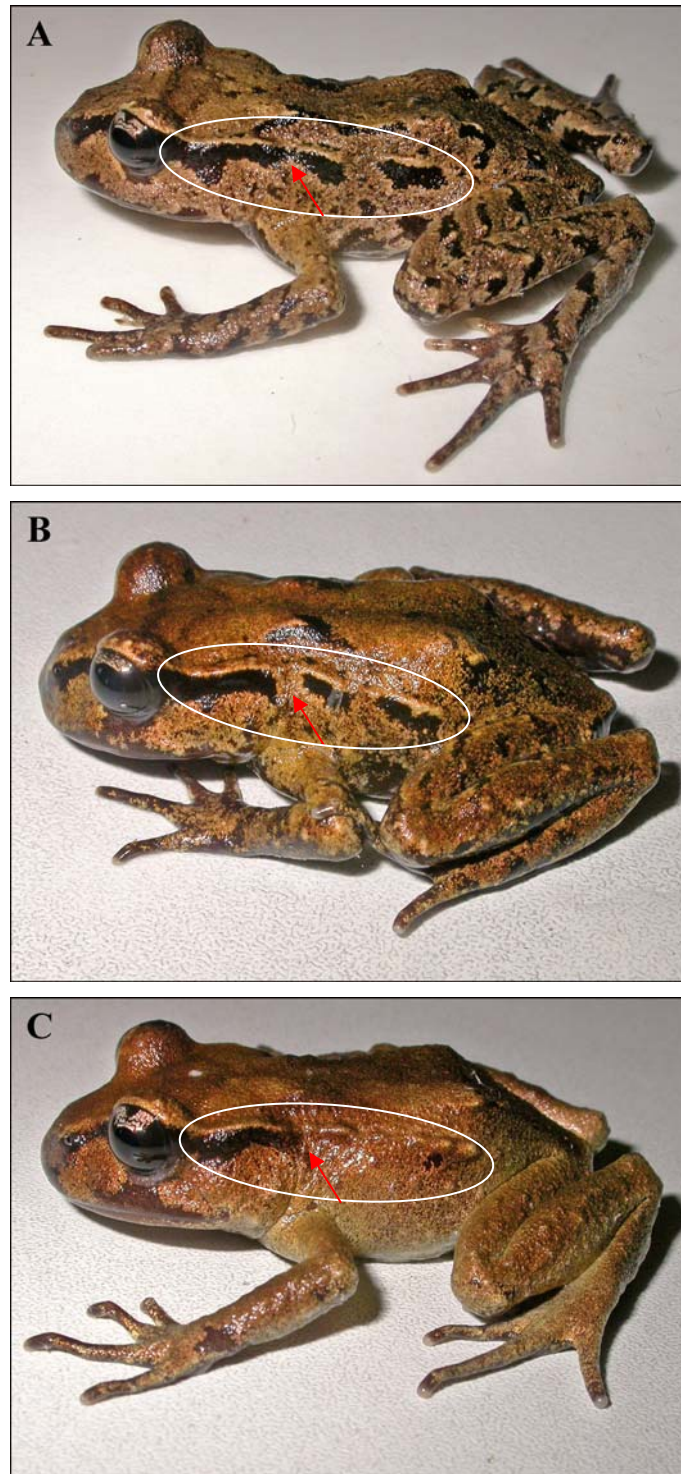


Figure 3 Dorsolateral view of left lateral line markings (circled) of Stage 2 for captive *Leiopelma hamiltoni*. A) Code A - Lateral line markings join at shoulder area (arrowed); B) Code B - Markings do not connect at point of shoulder (arrowed); C) Code C - Lateral line markings ends at shoulder position (arrowed).

- 1) Code A - The lateral line was complete all the way to just posterior to the frog's front leg (Figure 4A). For example, frogs in Group 1A would be coded Group 1AA.
- 2) Code B - The lateral line was broken before reaching the posterior side of the frog's front leg, but the darker pigment continued further down the lateral line of the frog (Figure 4B). For example the frogs in Group 1A would be coded Group 1AB.
- 3) Code C - The lateral line was incomplete, meaning the line did not reach the posterior side of the frog's front leg and did not carry on (Figure 4C). For example the frogs from Group 1A would be coded as Group 1AC.

This grouping technique allowed for the development of nine different sub-groups within each of the three main groups. With the use of only three different letters it is important that the same letter always represents the same pattern being observed. In this case the letter A always represented a solid unbroken line, regardless of whether it was on the face, or either lateral sides, ensuring the possibility of confusion is reduced.

Photographs of the 34 individuals within the sample population were given to four different human testers. The photographs were sorted into a folder in Adobe Photoalbum and four test subjects were given access to the photographs, and a set of instructions explaining each of the different stages of the classification process. Sample photographs were provided to illustrate the body mark being used. Each test subject used the different classification stages to place each of the frogs into the sub-groups. Their answers were recorded on an answer sheet and compared to the master list. None of the test subjects were involved in the development of the identification technique.

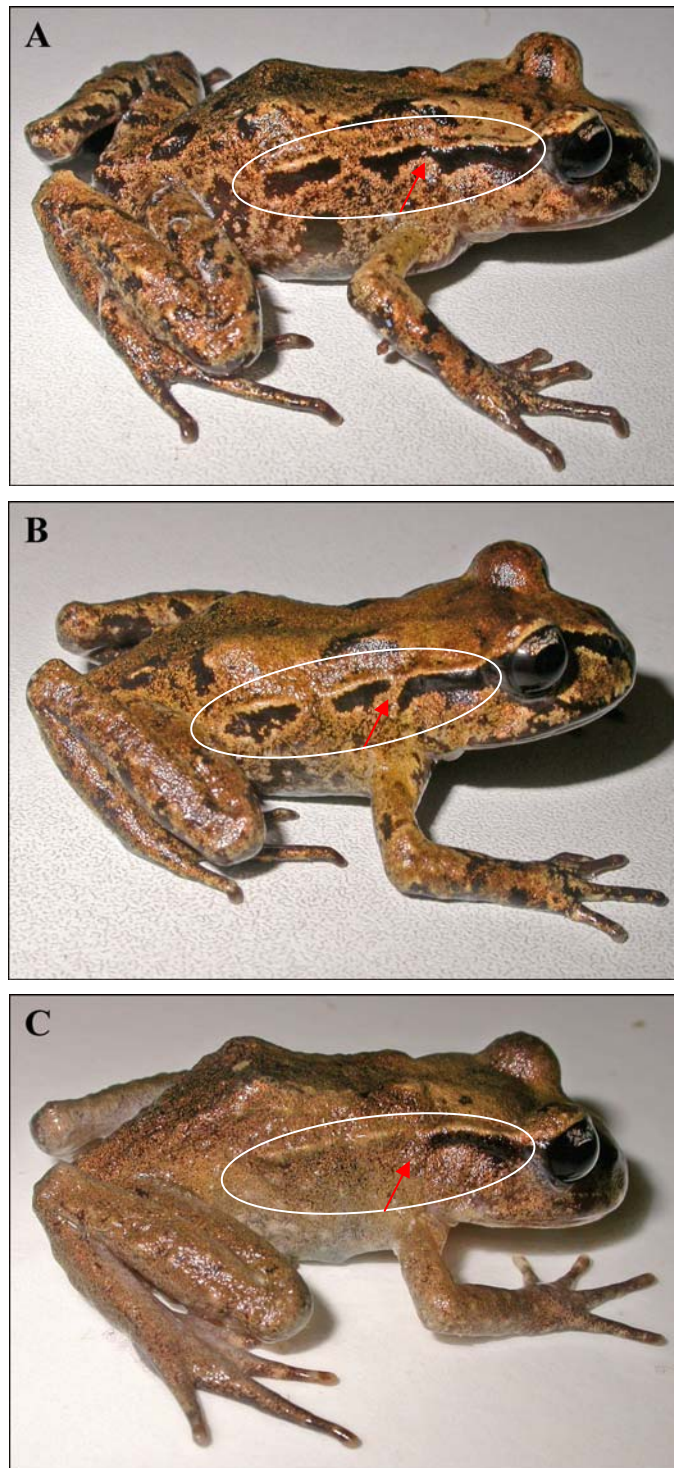


Figure 3 Dorsolateral view of right lateral line markings (circled) of Stage 3 for captive *Leiopelma hamiltoni*. A) Code A - Lateral line markings join at shoulder area (arrowed); B) Code B - Markings do not connect at point of shoulder (arrowed); C) Code C - Lateral line markings ends at shoulder position (arrowed).

The second stage was developing a technique that would allow for identification of recaptured animals. To test for recaptured animals, ten live frogs were selected from the 34 sample frogs. Four human testers used the classification method to place each live frog into the various sub-groups. Once the sub-group was established, a list of all possible frogs within the particular sub-group for the different frogs was given to each person. The live frog was then matched to the photographs in the sub-groups. The results from the testing of the frogs were scored on a scaling system. Four marks were given to each frog that was correctly identified on the first attempt, three marks after the second attempt and if a third attempt was required only one mark was awarded.

2.3 Results

The individual identification technique allowed the 34 captive frogs to be placed into a total of 27 different sub-groups.

The frogs were classified into three main groups based on the body marks on the facial region (Figure 2). Of the 34 frogs, five were placed into Group 1 (Table 4), 16 frogs were classified as Group 2 (Table 5) and the remaining eight were placed in Group 3 (Table 6).

Once all the frogs were classified into one of the three major groups, then each of the frogs was reclassified according to the darker pigmented line that followed the animal's lateral line (Figure 3 and Figure 4). Stage 2 classifications resulted in the five frogs into Group 1 being split into two smaller sub-groups. The frogs in Group 2 were divided into all three sub-groups available, but the majority were within Group 2B. Group 3 frogs were also split into two sub-groups (Table 6).

Table 4 Classification of frogs for Group 1. Group 1 criteria were the line on the inside of the eyes joined completely with the marking around the nostril region on the snout of the frog. Columns 1A-1C were the first sub-group (stage 2), classification based on lateral line on the animals left. The remaining columns were the division of the frogs after stage 3 classifications based on the lateral line on the animal's right side.

Group 1	1A	1B	1C	1AA	1AB	1AC	1BA	1BB	1BC
Frog 28		Frog 28						Frog 28	
Frog 5		Frog 5					Frog 5		
Frog 34		Frog 34							Frog 34
Frog 32		Frog 32						Frog 32	
Frog 7	Frog 7			Frog 7					

Table 5: Classification of the frogs in Group 2. Even after stage 2 classification the majority of frogs remained in the same sub-group.

Group 2	2A	2B	2C	2AA	2AB	2AC	2BA	2BB	2BC	2CA	2CB	2CC
Frog 12		Frog 12						Frog 12				
Frog 8	Frog 8			Frog 8								
Frog 4		Frog 4						Frog 4				
Frog 22		Frog 22						Frog 22				
Frog 1		Frog 1						Frog 1				
Frog 31		Frog 31						Frog 31				
Frog 14		Frog 14						Frog 14				
Frog 18		Frog 18						Frog 18				
Frog 17		Frog 17						Frog 17				
Frog 29		Frog 29						Frog 29				
Frog 25		Frog 25						Frog 25				
Frog 13		Frog 13						Frog 13				
Frog 10		Frog 10						Frog 10				
Frog 24		Frog 24						Frog 24				
Frog 27	Frog 27			Frog 27								
Frog 30	Frog 30			Frog 30								
Frog 2	Frog 2			Frog 2								
Frog 23		Frog 23						Frog 23				
Frog 16	Frog 16					Frog 16						
Frog 3		Frog 3						Frog 3				
Frog 37			Frog 37								Frog 37	

The individual frogs within Group 3 showed to have similar body for at both Stage 2 and Stage 3 as the other frogs allocated to the group, although the marks were different between the two stages (Table 6).

Table 6 Classification of Group 3. After Stage 3 classification the majority of frogs were allocated to the same sub-group.

Group 3	3A	3B	3C	3BA	3BB	3BC	3CA	3CB	3CC
Frog 9			Frog 9						Frog 9
Frog 26			Frog 26						Frog 26
Frog 20			Frog 20						Frog 20
Frog 33			Frog 33						Frog 33
Frog 6		Frog 6				Frog 6			
Frog 15		Frog 15				Frog 15			
Frog 21			Frog 21			Frog 21			
Frog 36		Frog 36				Frog 36			

The accuracy of classifying the frogs into the sub-groups ranged from 94% in stage 1 to 62% in stage 3 (Table 7). The accuracy of stage one varied from 94% to 76%. There was less variation in the accuracy of stage 2 with the accuracy being either 76% or 79%. Stage 3 classifications varied from 62% to 71%. However, if the accuracy of stage 3 classifications was calculated without taking previous classification errors into account, then the lowest accuracy rating was 73%, with the accuracy as high as 88%. The overall accuracy varied from 73% to 81%. When all test results were combined the grand total was 76% mean accuracy (Table 7).

Table 7 Photograph identification testing of *L. hamiltoni* (n=34) into categories by four human testers. The accuracy of stage 3 was tested separately, as well as a continuation from Stage 1.

Human Tester	Stage 1 classification (facial view) (%)	Stage 2 classification (left lateral line) (%)	Stage 3 classification (right lateral line) (%)	Accuracy of stage 3 marking only (%)	Overall accuracy (%)
1	76	76	65	88	73
2	76	79	68	88	75
3	94	79	71	82	81
4	85	76	62	73	75

The results from the testing of the frogs after the frogs already had been classified into categories were scored on a scaling system. Four marks were given to each frog that was correctly identified on the first attempt, three marks after the second attempt and if a third attempt was required only one mark was awarded. The number of frogs that were classified accurately on the first attempt varied from all ten frogs to only five frogs (Table 8). However, only one frog in one test required a third attempt to correctly classify the frog in the correct category and identify the correct frog. The overall accuracy of identifying a recaptured frog varied from 83% to 100% based on the scaling system according to the number of attempts required to correctly identify the frog. The grand total once all results were combined was 93% mean accuracy (Table 8).

Table 8 Results of photograph identification of frogs within categories. Four marks were awarded for each frog correctly identified first attempt, three marks for the second attempt and one mark for the third attempt.

Human Tester	# correct 1 st attempt (out of 10)	# correct 2 nd attempt (out of 10)	# correct 3 rd attempt (out of 10)	Total accuracy
1	8	2	-	95%
2	7	3	-	93%
3	5	4	1	83%
4	10	-	-	100%

2.4 Discussion

The more commonly used methods of marking amphibians for the purpose of individual identification involve techniques that are potentially harmful to the individuals (eg toe clipping) or expensive equipment (PIT tags). With the current decline of amphibian populations it is important to establish non-invasive methods for identifying individuals. The current method of individual identification for *L. hamiltoni* is by toe clipping. However, one of the more commonly accepted theories is that the declines are due to disease and the practice of toe clipping gives opportunistic pathogens an extra chance of infecting individuals. Toe clipping not only

leaves an open wound, but also causes stress due to handling (Coddington & Cree 1995) that may suppress the frog's immune systems long enough to enable pathogens to become established. There are also other injuries that can occur to amphibians while being handled (eg skin abrasions), which may provide more entry points for possible pathogen infections.

Individual identification based on natural or acquired markings has been used for various amphibian species (Newman, 1982; Loafman, 1991; Doody, 1995; Meyer & Grosse, 1997; Bradfield, 2004). There are many advantages to using an individual's natural markings as a technique for identification. The first of these is that once it has been ascertained that the markings being observed do not change with age or size of the animal, then this method is very reliable over long timeframes, unlike toe-clipping, which, even do not regenerate can lead to non marked animals can being mistaken as marked animals, due to individuals losing digits because of predation events or as a result of an injury (Doody, 1995 Gray *et al.* 2002).

An identification technique that has been used on individual *A. opacum* has involved counting the number of dorsum bars on each animal (Doody, 1995). Doody's method, once established, allowed individuals to be identified without comparisons to photographs being needed for confirmation. The use of counting the number of spots on each animal is not a practical method for *L. hamiltoni* as the pigmented patterns are not distinct spots, but are variable patches. However, using the various patterns of particular pigmented areas allows for individual identification. Due to the complexity of patterning of some of the frogs, photographs are always going to be needed to confirm the identity of various individuals.

Through the use of archival photographic records (Table 4, Table 5 and Table 6) it has been shown that there are certain markings on *L. hamiltoni* that do not change over time or as the frogs grow. This means that these markings can be used for identification purposes. Therefore the

use of these body marks provides a technique to individually identify *L. hamiltoni*. The body marks of interest are the markings around the eyes and snout, and the lateral lines on both sides of the animals.

The results obtained show that it is possible to use body marks of *L. hamiltoni* to identify individuals. It is possible to both classify the frogs into various sub-groups, and to re-identify individual frogs within the various sub-groups at a later date. Classifying each frog depending on the appearance body mark allows for the option of 27 different sub-groups to be established. The three different versions of each of the body marks used are, for example, in Stage 2 the darker pigmented line that runs along the animal's left lateral line, starting from behind the eye. This body mark is broken into three different sub-categories based on whether this line remains as one line until it is on the posterior side of the front leg (Figure 2), or if there was a gap in the line before the posterior side of the leg but continued further down the lateral line (Figure 3), or the line stopped before reaching the posterior side of the leg and did not continue (Figure 4).

Although this technique allows for 27 different sub-groups to be formed there are only three different body marks that are used: the facial region, the left lateral line and the right lateral line. Each of the different body marks can be classified one of three ways, depending on the appearance of the body mark. Once this technique is clearly explained with examples of all different versions of all three body marks this technique is very easy and straight-forward to use.

Once the frogs were categorised into sub-groups the mean accuracy of identifying individuals was 93%. The highest score of 100% was obtained by the human tester 4 (Table 8). However, this person is very familiar with the Maud Island frogs and is responsible for the daily care of the captive population held at Canterbury University, which indicates the accuracy of the technique increases as the human observer's familiarity with the species of the frogs increases. It

is very important that the frogs can be identified accurately once they have already been placed into sub-groups as this represents recaptures. If the technique were to be used to sample the same area over an extended period of time, then there is the need to be able to clearly say if a frog has been sampled before or if it could possibly be a new recruit to the area.

The *L. hamiltoni* technique can be used with live animals, photographs, or a combination of both. During the testing stages, the human testers were only given the opportunity to place the frog into one of the final sub-groups to ascertain the accuracy rate of each of the different classification stages. This is compared to Bradfield (2004) where if the classification of the frogs was unclear then the frogs could be placed into a primary sub-group, which was the group the frog most likely belonged in, but there was the option of placing the frogs into secondary groups. During the trialling stage of the technique one of the human testers placed one frog into a total of eight different groups, one primary group and seven secondary groups. If the frogs can be placed into multiple sub-groups then this could potentially lead to confusion, especially as the sample sizes in each of the groups become larger. By having the frogs placed into multiply sub-groups it increases the search time before each of the frogs can be correctly identified as each frogs would need to be checked against all possible sub-groups. The fact that on several occasions multiple frogs were classified into several groups may be an indication that the body mark patterning used was too complicated to allow for clear distinctions between groups to be made.

The technique developed by Bradfield (2004) for individual identification for *L. archeyi* uses four body marks that are classified as either continuous or discontinuous. The body marks used on *L. archeyi* were complex patterns due to the nature of the frog's markings, which could potentially become confusing to an inexperienced person or when working with large sample sizes. However, due to the various colour differences on the body marks on *L. archeyi* it is

possible to use colour as a back up method to confirm identification. By contrast, *L. hamiltoni* body marks are all the same darker pigmented colour and only brown shading of the skin pigment colouration varies between frogs

One of the major concerns with the technique developed for individual identification of *L. archeyi* (Bradfield, 2004) is that at this stage it has not been confirmed if the body marks of *L. archeyi* change over time or with the growth of the animal. Therefore, it is important to confirm that the body marks used in the identification technique are the ones that do not change or move over time or with the growth of the frogs.

Individual identification of *L. hamiltoni* will allow for more detailed research within the field. By identifying individuals by their body marks it reduces the possibility of mistaken identity of the frogs allowing detailed surveys of the number of frogs within a given area can be conducted without the risk of the same frogs being counted multiple times. More detailed studies with regards to *L. hamiltoni* home range and movement can be conducted. It is feasible that a day search will be able to identify the various retreat sites for each of the frog. This will give a precise starting point for each night which may allow for a clear indication of the distance the frog moves each night.

The results obtained from testing the identification technique for *L. hamiltoni* indicate that the accuracy rate increases with increased familiarity of the animal and the body marks being used. Bradfield (2004) came to the same conclusion with the technique developed for *L. archeyi*. Although this technique worked on a small sample size within a controlled captive environment, it needs to be tested further before saying it is an effective and reliable method of identification.

One of the main concerns with this method is the quality of the photographs. It is very important to avoid excess reflection from the animals when the flash is used. Any excess

reflection makes it difficult to clearly define the markings of interest. The focus of the image needs to be sharp, as any fuzziness of the picture can also make it difficult to clearly distinguish markings. The final aspect of the importance of photograph quality is the angles of the camera and position of the frogs. Some of the photographs used were on slightly differing angles, which increased the difficulty to compare similar markings.

One of the major limiting factors for individual identification is a method of storing the photographs. Although, the photographs used in this trial were in Adobe Photoalbum, the program had limitations with regards to how the program sorts and stores them. For example when using a digital camera each photograph is given a particular numerical code, and it is this code the program sorts the photographs by. It is possible to rename and move the photographs between folders but the program will also sort them by the numerical code.

The dark pigmented patterns on the skin of *L. hamiltoni* on both the lateral lines of the animal and on the facial region are stable over a timeframe of greater than three years, resulting in their suitability to be used in the development of a individual identification technique. It is through these body marks that a technique was successfully developed allowing for individual identification of *L. hamiltoni*.

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Chapter Three

Home Range

3.1 Introduction

Many vertebrates including birds (Hingrat *et al.*, 2004), mammals (Bonaccorso *et al.*, 2002; Broomhall *et al.*, 2003; Kalmler *et al.*, 2003; Monsnire *et al.*, 2003; Schmidt *et al.*, 2003; Taylor & Skinner, 2003), reptiles (Ferner, 1972) and amphibians (Ashton, 1975; Semlitsch, 1981; Kleeberger & Werner, 1982; Kleeberger, 1985; Muths, 2003; Watson *et al.*, 2003) have home ranges. Home ranges function as areas where important resources (e.g. food, nesting sites, and potential mates) are found within. Some but not all species defend their home range.

However, the definition of home range has changed over the years. Brown (1975) argued that an animal may have a home range but not actively defend some or all of it. Rather, an animal's home range can be considered simply as the area in which the animal normally lives and fulfils daily requirements. The home range of an animal does not include any migratory area, such as communal breeding sites. The area covered during emigration and dispersal events cannot be included in the area defined as its home range, as these behavioural events are not classified as everyday activities. The presence or absence of behaviours such as territoriality should not be used as an indication of an animal's home range while some animals will only actively defend various areas, for example, retreat sites.

Over the years there have been studies conducted on amphibian home ranges (Ashton, 1975; Semlitsch, 1981; Kleeberger & Werner, 1982; Kleeberger, 1985); however, many of these studies have included migration to breeding sites (Durham & Bennett, 1963; Rose, 1966; McVey

et al., 1981; Kleeberger & Werner, 1982; Stenhouse, 1985; Crump, 1986) which is not consistent with Brown's (1975) definition of area used to determine the size of a home range.

An animal may spend differing proportions of time within various sections of its home range; for example, this may vary depending on whether it is day or night (Brown, 1975). Areas within the home range may provide energy-efficient foraging sites, meaning that a high level of food can be found without the animal expending a lot of energy. Therefore, the animal may spend more time within these areas foraging (Test, 1954; Kleeberger & Werner, 1982; Gabor & Jaeger, 1995; Townsend & Jaeger, 1998) or the area may be used as calling or breeding sites (Wiewandt, 1969; Kleeberger & Werner, 1982; Heying, 2001). Alternatively, an area may serve as a retreat site (Test, 1954; Kleeberger & Werner, 1982; Kleeberger, 1985; Lang & Jaeger, 2000; Gauter & Maud, 2003; Watson *et al.*, 2003). If the animal is territorial, the retreat sites or breeding sites may be the areas where the individual animals exhibit territorial behaviours (Wells, 1977).

The species that do maintain home ranges fall into three categories; firstly many will actively defend these areas from all intruders and sometimes intruders are only individuals of the same sex (Lutz, 1960). The second category is the species that only defend certain aspects within their home range (e.g. breeding sites). Finally there are the species that have a home range but do not actively defend or deter conspecifics from within their home range.

Defending and maintaining a home range may function to give the resident exclusive access to nesting sites or resources, for example food or potential mates (Mathis, 1990; Mathis, 1991a; Jaeger, 1995) that are available within this area. There are costs associated with defending a home range (Mathis, 1991b); therefore the benefits need to be greater than these costs. There are ways that an individual can reduce these costs. If an individual had to continuously defend its

territory by fighting off competitors, then the cost both energetically and physically would be very high. However, through the use of pheromones or faecal markers, individuals can inform conspecifics of their territorial boundaries. The use of these chemical markers also potentially gives the intruder some idea of size and sex of the resident size (Mathis 1991b; Gauter & Miaud, 2003) and potentially enables the intruder to judge whether they are stronger than the resident, hereby avoiding unnecessary conflicts which may result in injury. Many species will defend their home range and place scent cues around the boundary to warn and deter unwanted intruders (Jaeger *et al.*, 1986; Horne & Jaeger, 1988; Mathis, 1990; Jaeger, 1993; Lee & Waldman, 2002; Gauter & Miaud, 2003; Waldman & Bishop, 2003), whereas some animals have a home range but do not actively defend or deter others from their area. The size of the home range can vary greatly depending on the size of the animal; however, this is not always a reliable method of determining the potential home range size (Kleeberger & Werner, 1982).

The environment and population density can have an impact on the size of an individual's home range. Kleeberger (1985) showed that both the density of the plethodontid salamander, *Desmognathus monticola*, within a given area, and the availability of cover objects affected the size of the home range. To show this Kleeberger set up three different treatments; treatment one involved developing low and high-density populations of *D. monticola*. Cover objects, such as rocks, were added in treatment two and in treatment three both cover objects and *D. monticola* were added. The size of the home range increased in treatment one only.

For this chapter a home range is defined as the area in which an individual may travel around in search of resources, including, but not limited to, food and potential mates, as defined by Brown (1975). For this chapter a home range is not confined to just the ground level. As

Leiopelma hamiltoni can be found in the vegetation (Bell, 1978), home range includes all vegetation, trees and branches above the ground of the area travelled by frogs.

3.2 History and Current State of Study Site

The study site was within the remnant forest on Maud Island. Maud Island is situated in the Tennyson Inlet of the Pelorus Sounds, Marlborough Sounds (Newman, 1982; Bell, 1995). Since 1867 the island was privately owned (Bell, 1995) and operated as a farm. The vast majority of forest was cleared and the island was heavily grazed. Approximately 16 hectares of forest (Figure 5) was left to protect the water catchment (Bell, 1982; Newman, 1982; Bell, 1995). This forest remnant provided the refuge for *Leiopelma hamiltoni*.

Kohekohe (*Dysoxylum spectabile*) and mahoe (*Melicytus ramiflorus*) make up the majority of the canopy on the less steep slope below 200 metres above sea level (a.s.l) (Crook *et al.*, 1971). Tawa (*Belischmidea tawa*), pukatea (*Laurelia novea-zelandiae*) and pigeonwood (*Hedycarya arborea*) are also found below 200 metres (a.s.l) (Crook *et al.*, 1971). Kohekohe and kawakawa (*Macropiper excelsum*) form the sub canopy and ferns such as *Arthropteris tenilla*, *Asplenium bulbiferum* and *Phymatodes scandens* are abundant at the ground level (Crook *et al.*, 1971). The dominant tree species for the canopy above 200 metres (a.s.l) is hinau (*Elaceocarpus dentatus*) and kamahi (*Weinmannia racemosa*) (Crook *et al.*, 1971).



Figure 5 View of the main forest area. Photograph was taken from just below Comalco Lodge. Study site is approximately 90 m above the forest edge.

Maud Island has remained free from introduced predatory species despite human occupation. Stoats have been captured on the island but none have managed to become an established population (Bell, 1982). Over years of human occupation the island forest was cleared to allow for the establishment of pasture for farming (Bell, 1995). A large portion of the island is still covered with pine trees, near Te Pakeka point (Figure 6). However, these trees are slowly being cleared as part of the island management plan.

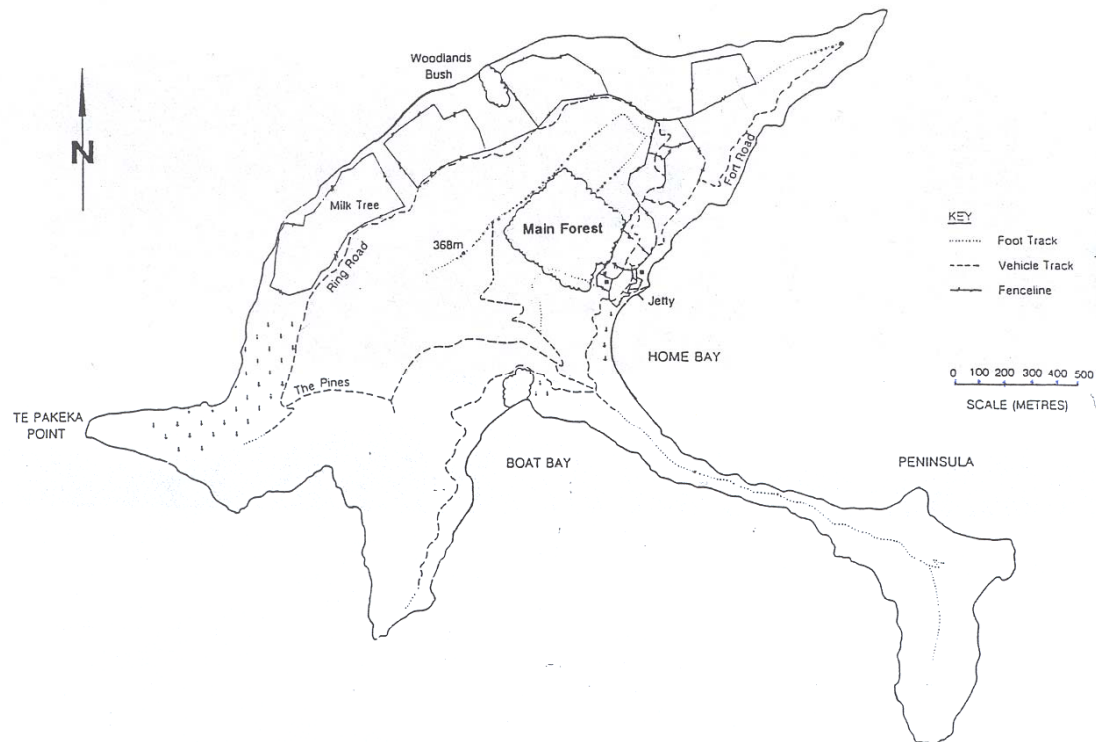


Figure 6 Map of Maud Island showing the island as it is currently. The main forest 16 hectare of remnant bush was left as part of the water catchment. The main forest contains the majority of the frogs. However there is another patch of remnant forest in Boat Bay, which contains a translocated population. (from Bell, 1995).

One of the first uses for Maud Island was to establish it as a breeding/refuge island for the remaining Fiordland kakapo, a critically endangered flightless parrot endemic to New Zealand. Disease remains a major threat to its frog population, although visitors to the island are monitored and the largest proportion of the island where the frogs are located is restricted to permit holders for scientific research only, although the frog population is spreading.

The New Zealand Department of Conservation currently, in its management plan translocates groups of individuals from the main population onto other islands that are free from introduced predators. These populations are intended to act as safeguards in case a disease or predators become established on Maud Island and decimate the main population. However, very little is known about this species; therefore it is very difficult to guarantee the success of translocation events. It is hoped that an increased knowledge of *L. hamiltoni*'s home range will

aid in the management of the population. As there are no confirmed morphological differences by which to distinguish the sexes, it is possible that determining the home range size of individuals may potentially allow for the different sexes to be distinguishable based on the size of the home range of individuals. The determination of an individual's home range is vital to ensure the success of future management plans and translocation events of this species, and potentially the other *Leiopelma* species.

3.3 Method

A grid was established in the remnant stand of forest within Strands Reserve, Maud Island (Figure 1, chapter 2). The grid was 180 metres above sea level (a.s.l) and was approximately 90 m from the forest edge. The grid diameter was 12 m long and 6 m wide (Figure 7). There was a buffer zone of two metres around the whole grid. Within the main grid 1 m square grids were marked out. Each of the smaller grids was allocated a code, consisting of a letter and a number for ease of referencing. Letters were allocated to the width of the grid and the numbers were allocated to the length (Figure 7). Therefore the bottom left corner was coded 1A.

The first 20 frogs found within the grid site were selected, regardless of the distance each frog was found from the previously selected frog. The SVL of the frogs was not considered when selecting frogs. At the time of selection the frogs' ventral snout-vent length (SVL) was measured to the nearest 0.5 cm. ventrally using a ruler and recorded. The 20 individuals selected were photographed for individual identification. The photographic views were dorsal, lateral sides, ventral, face and rump. The camera was 30 cm away from the frog and was on the same level as the frog, except for the dorsal view when the camera was directly above. Each frog also was toe-clipped with part of one toe being removed to be used as a backup system for confirmation of the

frogs' identification, ensuring the same frog was being tracked at all times. The location of the frog was recorded as well as the substrate or vegetation the frog was found on.

	2M	1M	A	B	C	D	E	F	M1	M
2M										
1M										
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
M1										
M2										

Figure 7 Grid lay out used for tracking home ranges of *L. hamiltoni* on Maud Island. The grid had a 2m buffer zone around it; grids coded 1M, 2M, M1 and M2 represented buffer zones. As the numbers on the grid increase, the grid was further into the forest.

The marked frogs were not observed for the first three days after they had been toe clipped, despite thorough searches of the grid site and other frogs being observed on those nights. The frogs also were tagged with a Twink® (correction fluid) code, which was either a letter or a shape (Figure 8) on the dorsal surface, which remained on the frog's skin for between 2-4 days

depending on weather conditions, after which time the Twink code was reapplied. Frogs could be identified without unnecessary handling by using the Twink mark and photographic records of their natural patterns together. Unnecessary handling of the frogs may have resulted in an increase in stress levels (Coddington & Cree, 1995) and possibly affected the frogs' tendency to emerge on subsequent nights.



Figure 8 Photograph showing a Twink code on a frog that was tracked within the grid site. Twink code lasted between 2 and 4 days depending on the weather conditions.

The grid was searched each night approximately one hour after darkness; the searches were conducted systematically by starting in one corner, with each smaller grid being searched before moving onto the next within the same column. The process was repeated until each smaller grid within each column had been searched. Grid searches started on the ground (Figure 10) before checking the vegetation which formed the lower canopy (Figure 9) within that grid. Each frog observed had its location recorded according to the smaller grid codes. The habitat the frog was in and the height above the ground were recorded for each sighting. Ventral snout-vent lengths (SVL) were recorded for each frog. Observations were recorded during the months of July, August and September 2003 and during January 2004. Observations were recorded regarding the canopy vegetation composition, seedlings and the location of rocks and logs, which

may have been used as alternative retreat sites. The location of the frogs was then entered into the computer program Homerange v2.1.3 (Huber and Bradbury, 1999).



Figure 9 View of home range grid site showing the lower canopy covering made up predominantly of young Nikau palms.



Figure 10 View of the home range grid site at ground level. The pink stakes in view in the centre of the picture are the stakes that were used to mark out the smaller grids within the main home range grid.

Data were analysed in two ways, the first of which used the minimum convex method (MCP) to give a non-statistical area of the home range based solely on the external observational points. The second method used was the harmonic means (HM) method. This involved making a statistical calculation based on mean distances between observation points. The HM method

calculated home range size based on both 95% and 50% probabilities that the frogs would be found within the calculated area. The home range size was then compared to the frog's SVL, and the time it spent above the ground in the vegetation.

3.4 Results

All observations of the frogs occurred at night. The frogs were observed within the grid site on either fallen logs, amongst the leaf litter, or off the ground on tree branches or on the vegetation. Occasionally frogs were observed on bare rocks. The frogs remained motionless unless disturbed. On some occasions a frog needed to be handled to have its twink code re-applied and to allow for confirmation of its identity. After a frog had been disturbed, it typically moved away in a walking motion by moving one foot at a time in foot, unless startled again in which case leapt out of the light. Frogs that were in the vegetation when disturbed leaped onto the ground and froze within the leaf litter. The location within the grid site where each of the marked frogs was found was recorded according to the letter and number of the smaller reference grid in which the frog was found.

All the grid sightings (the letter and number of the smaller reference grids) for each of the frogs were transformed into Cartesian co-ordinates and entered into the computer program Homerange (Huber and Bradbury 1999). The x y co-ordinates were then used by the computer program to generate the size of the home range for each of the individual frogs. The x y co-ordinates for two of the frogs were not entered into the computer program as less than four repeat sightings were made for each of them, as the limit had be set as at least four repeat sightings to reduce the possibility of inaccurate home range calculations. The program generated two different home ranges. The first was based on the minimum convex polygon (MCP) method. For

this the computer used the outer most data points (based on x y co-ordinates) to generate the smallest possible polygon shape and then calculated the area within the shape, giving the size of the home range. The second method that the computer program used was the harmonic means (HM) method. The results obtained from this method were a statistically based calculation determined by the amount of variation of the x y co-ordinates. Frogs F and J were excluded, as they did not move sufficiently to enable the computer to calculate a home range.

The MCP showed large variation in the size of the home range for each of the frogs (Figure 11). The home ranges of *L hamiltoni* show not only large variation in size, but also in the shape of the areas in which the animals travelled. The graphical representation of the home ranges (Figure 11) showed that frog H seemed to move the least, while the home range for frogs E and G were the largest according to the MCP.

Frog H had the smallest home range size of 0.5 m^2 . This is compared to the largest home range size, 25 m^2 , shown by frog E and G (Table 9). There was only 1 mm difference in the SVL of the frogs H and E with the frog E being the larger frog with a SVL of 39 mm, while Frog G's SVL was 43 mm. The frog with the smallest SVL of 37 mm was frog A, and its home range size was 16 m^2 . The number of repeat sightings for each of the frogs varied from five to 16. Frog H only had five repeat sightings while frog E had eight and Frog G had nine. Frog D had the most repeat sightings with a total of 16 and a MCP calculated home range of 9.5 m^2 .

The HM method was used to calculate the home range for all the frogs that were used for the MCP method. The computer program calculated the home ranges based on both a 50 % and 95 % confidence levels that the frog could be located within the calculated area (Table 9).

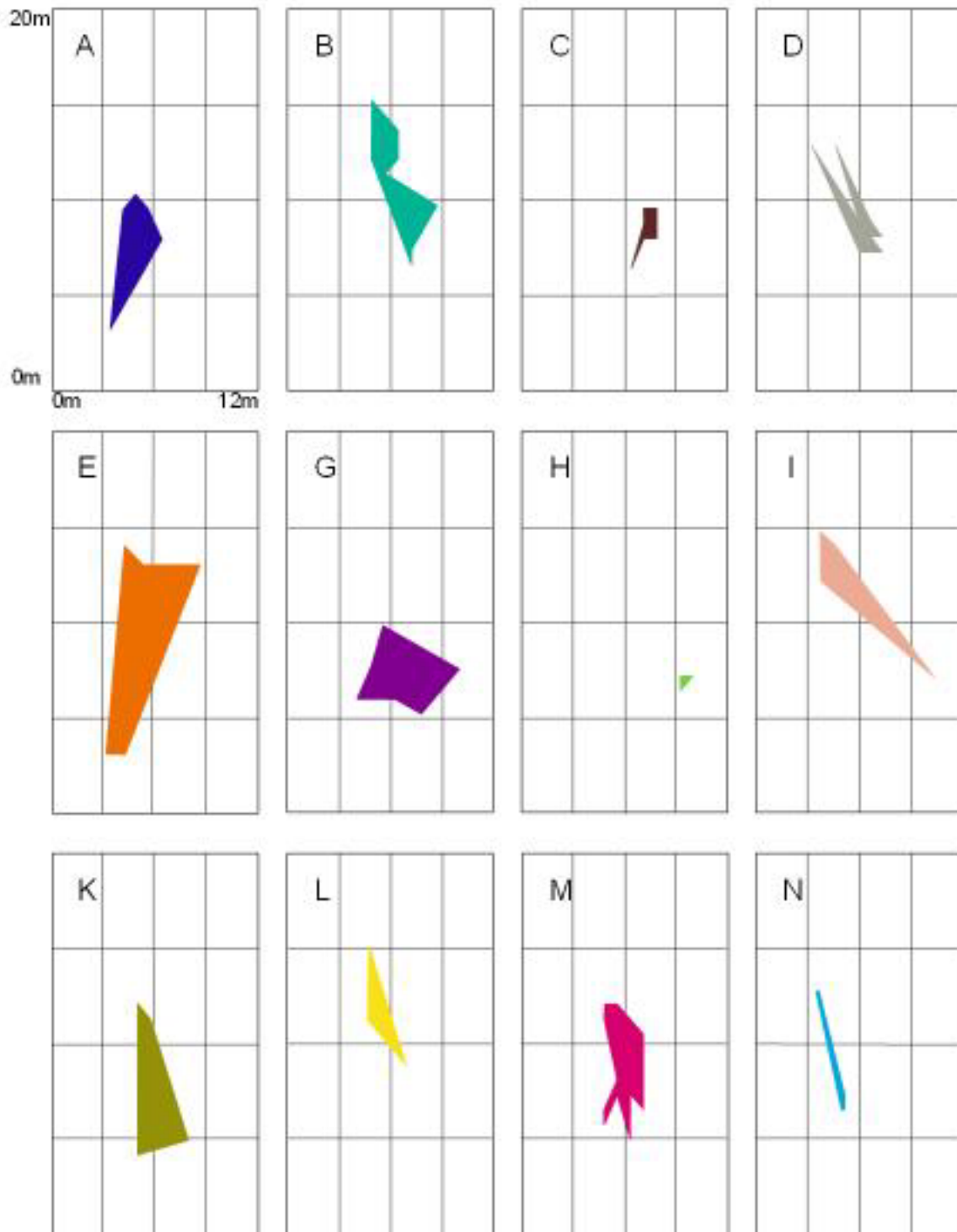


Figure 11 Graphical view of *L. hamiltoni* home range sizes according to the minimum convex polygon (MCP) method. All home ranges are shown as a 12 m x 20 m grid. The exact size of the home ranges was shown in Table 9.

The HM method indicated that the home range for all of the frogs was larger than suggested by the MCP method. The home range for frog H changed from 0.5 m² with the MCP method a 95 % probability of the frog being found within a home range size of 1.6 m² (Table 9). Frogs E and G's home ranges increased to 72.6 m² and 63.4 m² respectively. However, frog I showed the largest increase in home range size when the MCP method was compared to the home range size produced by using the HM method. The MCP indicated a home range of 11.5 m² that increased to 83.7 m² when the home range was calculated using the HM method (Table 9).

The centre of activity was calculated from the HM method to allow for skewed data. The centre of activity indicated where the frogs spent the majority of their time. The home range of frog H only overlapped the bottom corner of frog I's home range. The centre of activity for each of the frogs was calculated based on the HM home range size. The centre of activity showed the computer program's calculation of where the frog would spend the majority of its time. Several frogs potentially could have been found within close proximity to one another (Table 9). For example, frogs C and D could be found within one metre of each other, and frogs M and N could also be found within one metre of each other. However, one of the frogs of each pair had a smaller home range size (Table 9). For example, frog M's home range was 16 m², while frog N had a home range of only 1 m². Both home range sizes were based on the MCP method.

Table 9 Home range sizes for the frogs tracked. The minimum convex method (MCP) was home range size based on the area with the most external observation points. The harmonic means (HM) method home range showed 50% and 95% probability that the frogs would be observed within that area. The frogs in bold were recorded off the ground greater than 50% of the time. The centres of activity for each of the frogs are shown as grid co-ordinates.

Frog ID	SVL (mm)	Number of recaptures	Minimum Convex Method (m ²)	Harmonic Means Method (m ²)		Centre of Activity (grid co-ordinates)	
				95%	50%	X	Y
A	37	6	16.0	48.2	22.6	1M	7
B	39	9	18.0	73.3	32.3	C	10
C	38	7	2.5	10.6	4.5	E	6
D	38	16	9.5	55.1	20.7	F	6
E	38	8	25.0	72.6	31.0	B	11
G	43	9	25.0	63.4	26.5	A	2
H	39	5	0.5	1.3	0.6	M2	4
I	39	5	11.5	83.7	33.9	A	11
K	42	5	22.5	53.7	25.7	B	9
L	38	8	7.0	31.7	13.1	A	12
M	40	14	16.0	35.8	15.6	B	4
N	38	6	1.0	20.8	7.8	C	4

3.5 Discussion

Amphibians are declining on a global scale, and possible causes for the declines include habitat loss or fragmentation. Although there have been studies to show that some amphibian species are not affected by habitat loss and fragmentation (Silva *et al.*, 2003), others show that some species being affected. Johnston and Frid (2002) showed that the terrestrial Pacific giant salamander (*Dicamptodon tenebrous*) is affected, with the individuals that resided in the home range within the clear-cut area of the forest having smaller home ranges and spending more time in their sub-terrain burrows. These individuals were also more dependent on precipitation than the salamanders in the riparian buffer zones and the intact forested areas.

The higher dependency on precipitation may be a result of less leaf litter, which is able to retain moisture longer. The lack of litter also may mean that the food resources within these areas could be greatly reduced. Combined, the possible decrease of food within the clear-cut home range areas and a smaller home range may mean that these salamanders are not able to obtain enough prey items, or that the prey items that they are catching are of insufficient value (Gabor & Jaeger, 1995). Over time this may result in a shorter life span. Alternatively, it may be that it is the younger salamanders that are in the clear cut areas and if their nutritional intake is decreased, then this may affect their fitness, which could be detrimental to the long-term survival of the population or species.

The MPC produced home ranges that are based on the smallest possible polygon shape that can be generated by using all the outer most data points. However, one of the problems with the MPC method is that the number of recorded locations of the individual determines the area, which means that with more recorded sightings the size of the polygon and ultimately the home range may change. This may explain the reason for the home range of frog H that only has five repeat captures has a home range size of 0.5 m^2 ; however frog K counter-acts this explanation. Frog K has the same number of repeat captures yet has a home range of 22.5 m^2 . The number of repeat captures of *L. hamiltoni* is small, with the majority of frogs having less than ten repeat captures. This compares to many other studies where the repeat sightings are over 100 (Laiolo *et al.*, 2001; Osterwalder *et al.*, 2004). Laiolo *et al.* (2001) showed that the minimum convex polygon gave the largest home range size; however, their smallest number of repeated recordings for the same individual was 243. This meant that there were a far greater number of points for the polygon to be based on, which may affect the accuracy of the size of the home range.

As the frogs were not constantly tracked for the duration of observation nights, it is possible that not all of the frogs' home range was recorded. Moreover, the frogs' locations were only recorded if they were found within the 6 m by 12 m grid. Therefore, there was potentially a high probability of the frog not being recorded, even if it was out on that particular night, due to the fact that they may have moved beyond the grid boundaries. For this reason it was important to use a statistical program to calculate a home range area from the recorded data points. The HM method was used for this. It is a statistically based program that is able to calculate predictions of home range size based on the x, y co-ordinates. Home range areas were calculated based on both 50% and 95% probability the individual would be found within that area.

The home ranges for all the frogs were larger with this HM method, when compared to the MCP. However, as shown by Laiolo *et al.* (2001), the HM method produces a home range prediction that is, on many occasions, less than half the area shown by the minimum convex polygon. This suggests that the home range of *L. hamiltoni* may be larger than the results shown.

The graphic representation shows that there was a large variation in not only the size of the home range but in the areas in which these frogs were observed. The centre of activity was calculated from the HM home range size and showed that some frogs were found within one metre of each other. Frog M and N were found to have a centre of activity within 1 m of each other. However, the home range of frog M was 16 m² while frog N had a home range of 1 m². It could be that the size of the home range is determined by the sex of the frog. Frog M had a SVL of 40 mm while frog N's SVL was 38 mm. As neither of the frogs have a SVL that clearly can be classified as female, it is not possible to say definitively that sex is a determining factor, but this may represent an example of one sex overlapping several home ranges of the opposite sex. It is possible that both sexes may obtain advantages by having a larger home range that overlaps

several frogs of the opposite sex. As it is the males that are believed to brood the eggs, it is possible that the male may be able to obtain an advantage during breeding season by having access to multiple females within his home range. However, as the eggs produced by the female are large, the female has invested a lot of resources into the eggs and therefore she could be selective towards a particular male to guard her eggs. If this is the case then the female may gain the greatest advantage by having the larger home range and having access to multiple males.

The centre of activity may indicate the location of the frog's retreat site. The close proximity to another frog may be due to a limitation of suitable retreat sites. However, it is also possible that the frogs would not travel the entire area of their home range each night due to the small number of repeated sightings, the calculated centre of activity for each frog discussed in this chapter may not be a true indication of the frogs' centre of activity.

Previous studies by Waldman and Bishop (2003) indicated that the frogs showed a greater preference for the substrate that they had marked themselves compared to substrate marked by a frog collected further away. The frogs showed no preference when the substrate had been marked by a neighbour. The large extent of the overlap of home ranges may explain findings indicating that the frogs are familiar with the odour of the neighbouring frogs, as they may come in contact with the marking odours regularly, whereas if the odours from the faecal pellet which is used as markers, is from another frog beyond its own home range, then this odour would be unfamiliar. It is possible that the frog's relatedness to neighbouring frogs is determined by distance. This also may mean that closely related individuals may also share home ranges. The relatedness of neighbouring frogs also may be the explanation as to why some frogs have a centre of activity within 1 m of another frog (frogs M and N), yet other frogs have a centre of activity that is over 2 m from the next closest frog. For example, frog H's centre of activity is over 2 m away from frog

G, which is the frog whose centre of activity is the closest. However, if the frog's centre of activity is an indication of relatedness it is possible that frog H was on the boundary between one related group and the next.

Alternatively it may be that within that area, there are a few individuals of one sex and their home range overlaps many individuals of the opposite sex (Ferner, 1972; Mathis, 1991b; Bonaccorso *et al.*, 2002; Hingrat *et al.*, 2004) . It is possible that the home range size or the amount of overlap with other home ranges is affected by the sex of the individual frog. However, due to the difficulties in determining the sex of each individual frog, and the fact that there is little variation in the SVL of the frog observed here, it is difficult to predict how sex and home range size are related.

For this reason it is important that research into the home range of *L. hamiltoni* is taken to the next stage, which would involve developing a technique that will enable individual movements to be tracked continuously. The unfortunate aspect with regards to this is that the animal's small size eliminates the use of radio tracking devices, as currently they are too large. Alternatively, it may be necessary to track animals manually. The major hindrance to this method is that the animals can be inactive for long periods of time, and their natural defence mechanism of freezing when they feel threatened may prevent individuals moving if an observer is nearby. As these animals are nocturnal the easiest way of finding them is by the reflection of their eyes from a torch. However, as sudden change in lighting experienced by the frogs may affect their behaviour, this method may prevent the animal from exhibiting natural behaviour, and therefore give a false indication of its home range. It is possible to use infrared to observe the animals; however, due to the size of the frogs, and the area the HM method indicated as the size of the home range, it is not possible to set up a camera and track the animals. Therefore, the camera

would need to be manned and moved with the frogs, and if the frog senses movement then it may induce them to the defensive behaviour of freezing, which again would not give a true indication of the frogs' movement and home range.

Knowledge of the individual's home range is very important to enable successful management of various populations. Also one of the main management strategies for the Department of Conservation is to translocate groups of individuals from large successful populations to alternative predator-free islands. However, to ensure that sites chosen for translocation events fulfil the requirements of both the establishing individuals and the long-term sustainability of the population, the home range requirements need to be considered. The knowledge of individuals' home range requirements will also aid in the management of current populations. This knowledge can also form the baseline of either future research or management plans of other *Leiopelma* species.

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Chapter Four

Photographic Record To Monitor Population Health

4.1 Introduction

Amphibian population declines have in recent years become a global phenomenon. Many hypotheses have been proposed as to the reason for these declines: UV and other environment factors (Laurance, 1996; Lips, 1998; Pounds *et al.*, 1999; Blaustein *et al.*, 2001; Kiesecker *et al.*, 2001), human impact in the form of chemical pollution (Hecnar, 1995; Laurance *et al.*, 1996; Lips, 1998; Harris *et al.*, 2000; Davidson *et al.*, 2002; Saura-Mas *et al.*, 2002; Brown Sullivan & Spence, 2003) and habitat destruction (Tocher, 1996; Chazal & Niewiarowski, 1998; Johnston & Frid, 2002; Silva *et al.*, 2003), introduced predators (Drost & Fellers, 1996; Kiesecker, 2003), chytrid fungus (Crawshaw, 1997; Daszak *et al.*, 1999; Bosch *et al.*, 2001; Muths *et al.*, 2003) and other diseases (Esch *et al.*, 1997; Kiesecker & Skelly, 2000; Maksimowich & Mathis, 2000; Warkentin *et al.*, 2001; Johnston *et al.*, 2002; Pessier, 2002). In some cases, population declines have occurred gradually over several years (Richards *et al.*, 1993; Trenerry *et al.*, 1994; Drost & Fellers, 1996; Lips, 1998), and some species may have become extinct (Crump *et al.*, 1992; Richards *et al.*, 1993; Drost & Fellers, 1996). Unfortunately, it is difficult to find the carcass of a frog, and especially difficult within the short timeframe before the animal begins to decay or is destroyed by scavengers. This results in many unanswered questions as to why particular populations disappear. It is important to find a method for monitoring populations while there are still individuals alive in the wild as this would increase the possibility of an early detection of population declines and decrease the possibility of the population or species becoming extinct.

Monitoring populations of any one species may reveal critical data are applicable to other species. Many different techniques are available for disease detection within a population. However, many of these techniques require an animal carcass or at least toe clips (Berger *et al.*, 2002) or sloughed skin (Nichols *et al.*, 2001). There is the need to develop a non-invasive method of monitoring the health of animals on an individual level and a population level, and photographs are a potential solution. The advantage of photographs is that, once they are properly categorised, they reduce the possibility of observational variation both among observers and within observers.

The concept of using photographic records as a method of monitoring a species health status is not new. For example, Thompson and Hammond (1992) used photographs to monitor the health of a resident dolphin population in northeast Scotland. This population was used because skin lesions were noticed on photographs that were used for a previous individual identification study. The lesions were broken into three different categories, two of which had been observed in a captive population and could be linked to a disease.

Wright and Zamudio (2002) used photographs as a tool for monitoring a population health. By comparing the asymmetry of the colour patterns of *Ambystoma maculatum*, a salamander they showed that asymmetry could be used as an indicator of a population's health and fitness. This was achieved by measuring the asymmetry of the colour pattern of a disturbed population with a relatively undisturbed population, and by using museum samples of the now disturbed population. Wright and Zamudio photographed both live and preserved specimens and measured the size of the spots on each side and compared the difference in absolute values for each side. The snout-vent length (SVL) of each individual was used as an index of body size.

In New Zealand there are only three remaining extant endemic frog species, all of which are protected. The entire worldwide population of *Leiopelma hamiltoni* is restricted to three islands within the Marlborough Sounds region of New Zealand, with the majority of the population being on one island (Newman, 1982; Bell, 1994; 1995; Bell *et al.*, 1998). Potentially, any disease outbreak could result in dramatic population declines from which recovery may not be possible.

All *Leiopelma* species are susceptible to high stress levels (B. Waldman, personal communication), suggesting that regular and prolonged examinations are risky. The handling time required to obtain a skin sample may, in many cases be longer than is a safe duration before the animal becomes highly stressed (Coddington & Cree, 1995). The current *Leiopelma* population on Maud Island was observed with denuded (no visible skin pigmentation) patches on their body during January 2003 (B Waldman, personal communication). Initially these patches were most commonly found on the face, lips and throat region. However, more detailed observations revealed these patches were commonly found on the ventral area of the frogs. Less frequently the denuded patches were observed on the legs and feet. Other symptoms included large areas of the throat region being bright red in colouration. These symptoms were first observed in individuals that had recently arrived at the captive facility at the University of Canterbury. Further observations from photographic records from the January 2003 visit to the island indicated that frogs in wild populations were displaying these lesions.

4.2 Methods

Two different techniques were used for monitoring methodology. The first method involved taking photographs of individuals within the present population and comparing these to

photographs taken of members of the same population by Don Newman, Department of Conservation, between 1976 and 1983, during the months of March, May, September, and November. The past population photographs were taken from two different study sites, Grid 1 and Grid 2 (Newman, 1982; Bell, 1995) (Figure 1, chapter 2), whereas the photographs of the present population were taken at these two sites, as well as at my home range grid site, site H, (Figure 1, chapter 2) which was 50 m higher into the main forested area than grid 1. The photographs were taken during the months of January, July, August and September 2003, and January and February 2004. Both sets of photographs included close-ups of each subject's face, and both the right and left lateral view of the frog from snout to vent. A Nikon Coolpix 4500 digital camera was used to take the photographs of the present population. A flash was used when taking all of the photographs. The camera was positioned 30 cm away from the frog and was on the same level as the frog, except for the dorsal view when the camera was directly above the frog. The camera was in macro mode for all the photographs. The photographs were taken both by night in the field and during the day in a laboratory. All the photographs were downloaded into the computer program Adobe Photoalbum Version 2.0 and labelled according to the month the photographs were taken and the health condition of the frogs. The past photographs were categorised according to the date when the frogs were photographed and the study site at which the frogs were found (Figure 1, chapter 1).

Two aspects were compared between the past and present population. First observations needed to be made as to whether different size classes varied with respect to the frequency of symptoms. The size of each individual was based on the snout-vent length (SVL). The second aspect compared was whether the frequency of frogs observed with the symptoms of lesions had increased. Comparing the past photographs with the present lesions was used to answer both

questions. Initially only the photographs of the face and from both lateral views of the present population were used when comparing between population years, to allow for consistency of data available for all population samples. However, a second comparison was made using the ventral view of photographs from 2003 and 2004 to determine whether the percentage of the population was higher than initially shown.

To compare the sizes of lesions over time, body marks on the frogs were used to calibrate measurements. The body marks were the darker pigmented patches on the frogs (refer to methodology in chapter two). Once the scale was set for the photographs, a line was drawn around the area of the lesion using ImageJ to achieve an area reading. ImageJ is a public domain Java image-processing program, based on NIH Image for the Macintosh. Analysis was conducted using an Apple Macintosh computer. This method was used for the years between 1976 and 1983, and between 2003 and 2004 and the average area was obtained for each year.

To allow for change in measurements of the original frog with that of the subsequent photographs a ratio was obtained. Measuring both the lateral views with a ruler calibrated in mm from the vent to the tip of the snout gave the SVL of the frog in the photograph. This number was then divided by the known SVL giving a ratio number (refer to methodology of chapter two). Once the accuracy of the computer program was ascertained for measuring the frog's SVL, the next step was to determine the accuracy of the lesion area. This was done by using the known SVL to calibrate the line of the SVL of the photograph. A line was drawn around the outside of the lesion. In cases in which the frogs had multiple lesions, each lesion was measured separately and then the area of all the lesions was totalled. The computer determined areas were then compared to manually obtained results. To obtain the areas of the lesions, a sheet of graph paper was photocopied onto a sheet of transparent over-head projector (OHP) paper. The photocopied OHP sheet was placed onto of the original sheet of graph paper to ensure the squares were not

distorted during the process of photocopying. The OHP sheet was then placed on top of the original hard copy photographs taken by Don Newman, between 1976 and 1983. The number of squares that were filled with the lesion was counted. This number was then multiplied by the ratio for each of the frogs. The ratio was obtained by measuring the photograph and dividing this number by the known SVL. For some of the frogs the ratio was slightly different depending on which lateral view was being compared; therefore the highest ratio was used. These manually obtained lesion areas were compared to the computer generated results.

Table 10 Example of results comparing the manually obtained area of the lesions with that of the area obtained by the using the computer program ImageJ. The number of squares were multiplied by the ratio of the frog to account for the difference in size of the original frog and the developed photograph.

Frog	Number of squares.	Number of squares X ratio	Calculated manual area (mm ²)	Computer area (mm ²)
EJ20	6.00	24 sq X 0.57	13.68	13.96
EJ32	3.00	12 sq X 0.51	6.12	4.58
EJ52	8.00	32 sq X 0.52	15.60	16.19
EJ67	6.00	24 sq X 0.53	11.66	11.34
EJ81	6.00	24sq X 0.55	13.20	14.25
EJ9	12.00	48 sq X 0.62	29.26	31.39
OH16	5.00	20 sq X 0.53	9.54	7.67

The second method was adapted for captive individuals that were already displaying systems of potential diseases in the form of denuded patches, areas of thinned skin or areas of intense red colouration. Five frogs were monitored over an eight-month period. The frogs were originally found in the field (on Maud Island) and were taken into the captive facility at Canterbury University. Each of these frogs was originally photographed in the field. Upon arrival at the captive facility each frog was placed into a separate airtight container (320 mm long, 210 mm wide and 105 mm deep). The containers were made of a transparent polypropylene. In each

container, four paper towels, two damp and two dry, were added. The dampened paper towels were wetted with distilled water and all excessive water was squeezed out. The containers were cleaned weekly and the frogs were feed weekly.

The diet of the frogs was fruit flies (*Drosophila melangaster*), wax moth larvae and adults (*Galleria mellonella*), small crickets (*Teleogryllus commodus*) and small locusts (*Locusta migratoria*). This diet was not aimed to mimic that in the wild. It was, instead adopted as a way of ensuring that the captive frogs' diet contained essential nutrients and minerals. The most abundant prey taxa on the island, in order of abundances levels, are Collembola, Acari, Coleoptera, Amphipoda and Diptera (Bell, 1995).

The frogs were housed in a temperature-controlled room (daytime temperature set at 13 - 15° C and the night temperature changed to warm at 11 - 13° C during the summer, and 11 – 13° C during the day and 10 – 12 ° C during the winter). The photoperiod cycle was controlled with variations between summer and winter. During the summer cycle, the lights were on from 0700 hours until 2100 hours. During winter this changed to; on at 0600 hours and off at 1800 hours. The frogs were photographed for the second time six weeks after arriving, and then photographed for a third time a month later. The frogs were then photographed for a fourth time eight months after they originally arrived at the captive facility. These photographs were then compared, and the changes in the lesions or visible symptoms recorded. Observations included changes in the size of lesions, colouration and appearance of new lesions. The same methodology that was used to compare the changes in the lesions over time was used to monitor the change in size of lesions for each individual.

4.3 Results

Over the years, there was a gradual increase in the percentage of the population showing lesion symptoms, but 1976 and 1979 did not follow this trend (Figure 8). In these years there was a larger percentage increase of frogs showing lesion symptoms comparing the percentage

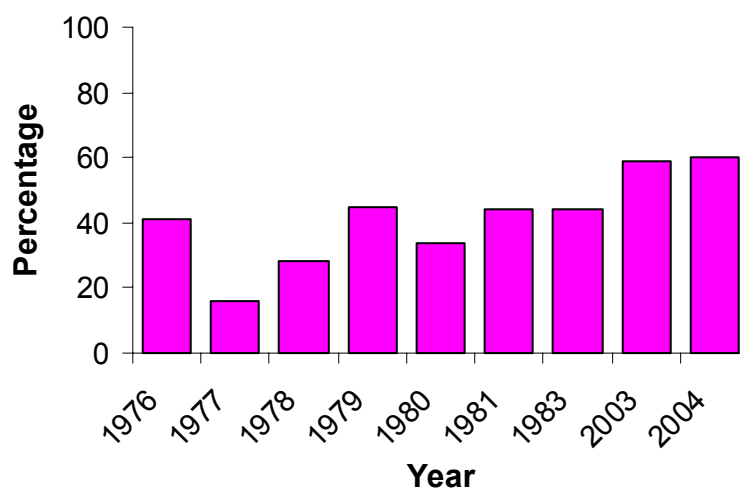


Figure 12 Percentage of the population during 1976 until 2004 showed lesion symptoms on the face and lateral regions.

of frogs that showed lesions for 2003 and 2004 (Figure 12), taking into account only the symptoms visible on the face and lateral regions, with the percentage of frogs observed with lesions when the ventral regions of the frogs were included (Figure 13). Large increase in the percentage of frogs with lesions were note (Table 11). There was an increase of 7% for 2003 and 22% in 2004.

Table 11 Chi-square tests of increase of number of observed frogs showing lesions for each year. Chi-square = 30.124, Degrees of freedom = 8, P value = 0.000.

	Year	1976	1977	1978	1979	1980	1981	1983	2003	2004	Total
With lesions	Observed Value	9	5	11	24	13	13	43	65	31	214
	Expected Value	9.97	14.06	17.68	24.03	17.23	13.15	44.43	49.87	23.58	
Without lesions	Observe Value	13	26	28	29	25	16	55	45	21	258
	Expected Value	12.03	16.94	21.32	28.97	20.77	15.85	53.57	60.13	28.42	
Total		22	31	39	53	38	29	98	110	52	472

The increase in the number of frogs observed each year with lesions is not a result of increased sampling in some years compared to other years as shown by the chi square test (Table 11).

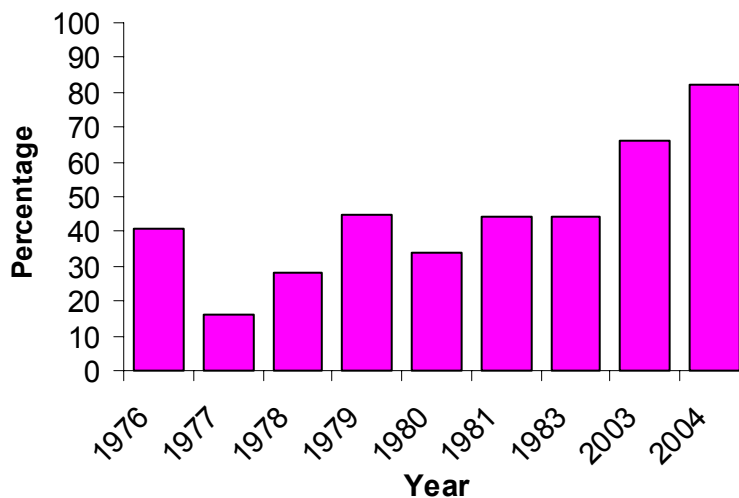


Figure 13 Percentage of the population observed from 1976 to 2004 showing lesion symptoms on the face and lateral regions. 2003 and 2004 observations also included lesion symptoms observed on the ventral regions of the frogs.

Frogs with the larger SVL were more susceptible to lesions (Figure 14). The lesions started to be detected in frogs with a snout-vent length (SVL) greater than 26mm. The frogs with snout-vent

lengths of over 46mm showed the greatest percentage of individuals with lesions, 78 % of the population sampled were recorded as having lesions.

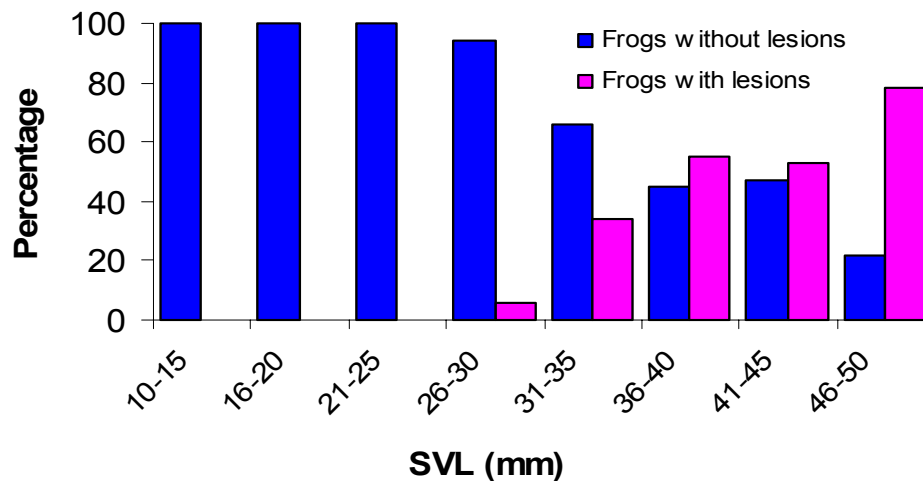


Figure 14 Percentage of observed population between 1976 and 2004 showing lesion symptoms on the face and lateral regions, as a function of snout-vent length (SVL).

The chi-square analysis shows that there was an increased likelihood of the frogs with a larger SVL being found with lesions than the smaller frogs when placed into SVL classes (Table 12), with a P value of 0.000.

Table 12 Chi-square results from the large SVL frogs being more likely to be found with lesions. Chi square value = 74.13, Degrees of freedom = 7, P = 0.000.

	SVL Classes (mm)	10-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50	Total
With lesions	Observed Value	0	0	0	1	12	89	66	14	182
	Expected Value	10.21	7.55	6.66	7.10	15.54	71.91	55.04	7.99	
Without Lesions	Observed Value	23	17	15	15	26	73	58	4	228
	Expected Value	12.79	9.45	8.34	8.90	19.46	90.09	68.96	10.1	
Total Observed		23	17	15	16	35	162	124	18	410

There was a gradual increase in lesion area until 1980, after which time the average area of lesions decreased for 1981 and 1983 (Figure 15). During 2003 the trend in decreasing average lesions continued if ventral view was excluded. With the inclusion of ventral lesions both 2003 and 2004 showed the large increases in the average area, 20mm^2 for 2003 and 45mm^2 for 2004. All the frogs sampled were above 36 mm SVL, which put them all into the top three size classes for the percentage of frogs recorded with lesions (Figure 14).

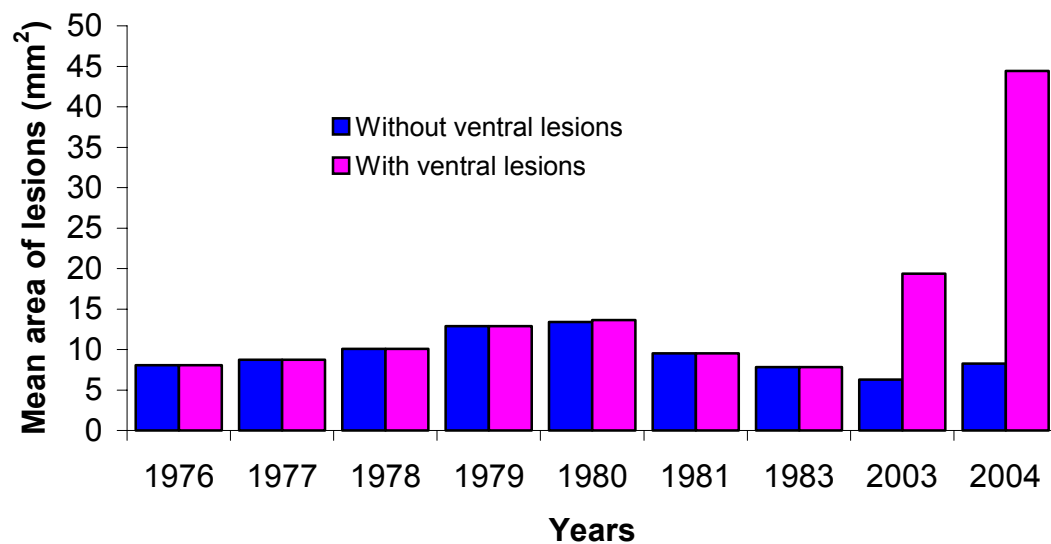


Figure 15 Mean area of the lesions found on the frogs for the years monitored. All the SVL were above 36 mm which fall into the top three size class for frequency of lesions. Data for the area of the ventral lesions were only available for 2003, 2004, and one frog in 1980.

To allow for greater comparisons between years ratios, were obtained based on the average SVL of the frogs sampled and the mean area of the lesions. The area of lesions was broken into two different categories, with the ventral view only being included in the second category. Without including the ventral view, 1978 through until 1980 had the highest ratio, while 2003 had the lowest lesion area to SVL ratio (Table 13). However, once the ventral view was included, 2004 had the highest ratio and 2003 was the next highest.

Table 13 showed the mean area of lesion (mm²) for each year. Only 2004, 2003 and 1980 lesion areas had changed once the ventral view was included.

Year	Mean SVL (mm)	Mean Area Lesion (mm ²) – Without Ventral	Ratio	Mean Area Lesion (mm ²) – With Ventral	Ratio
1976	39.58	8.07	0.20	8.07	0.20
1977	39.53	8.73	0.22	8.73	0.22
1978	38.65	10.08	0.26	10.08	0.26
1979	39.63	12.87	0.32	12.87	0.32
1980	42.83	13.39	0.31	13.63	0.32
1981	37.61	9.52	0.25	9.52	0.25
1983	38.72	7.83	0.20	7.83	0.20
2003	40.65	6.28	0.15	19.36	0.48
2004	41.13	8.25	0.20	44.43	1.08

Five sick frogs were collected from Maud Island in 2003 and were monitored to assess the ability of the animals to possibly cure themselves. The symptoms included sores on the side of their body, large areas of the skin of the throat region being unusually red, and large areas of denude skin pigmentation, which for this study these have been described as lesions. One frog also appeared to have one eye that had sunk into the socket.

The condition of the frog with the sore on its side deteriorated initially, with the sore developing into a large denuded patch. The skin at this site became very thin, and upon closer examination, the skin was transparent (Figure 16). The affected area measured 4 mm² when it was found in the field. This area increased to 28 mm² by the time the second photograph was taken, which was the largest size the affected reached. The area then showed signs of improvement. By the fourth photograph the affected area had become reduced to two small areas measuring 1 mm² and 3 mm² (Figure 16). The second frog showed improvement within the first two months of captivity (Figure 17). The bright red colouration on the throat decreased after arriving at the captive facility at the University of Canterbury. By the second photograph there was a noticeable difference in the intensity of the colouration, and by the third photograph the

redness was no longer visible. The remaining denuded patches on the throat measured 17 mm² and 7 mm². The denuded patches showed little change until the fourth photograph was taken at which time the denuded patches had decreased of a size of 7 mm² and 5mm² (Figure 17). The third frog was recorded with blisters on its ventral surface and the affected area fluctuated over time (Figure 18). Initially the affected area measured 54 mm², which decrease to 36 mm² at the next recording. However, by the third photograph the affected area had increased to 59 mm². The measurement from the fourth photograph showed that the affected area had decreased again and now measured 32 mm² (Figure 18). The fourth frog had one of its eyes that appeared to be permanently sunk into its socket (Figure 19). This frog showed very little change during the first three photographs; however by the fourth photograph the sunken eye was clearly visible and showed little variation from the frogs other eye (Figure 19). The final frog had a damaged eye and appeared to be totally blind in one eye (Figure 20). The condition of the eye did not change with time. The frogs showed little change in weight.

Table 14 Mass of the five sick frogs that were being monitored at the captive facility at the University of Canterbury.

Frog	15 th September 2003		12 th November 2003		Change In Mass (g)
	SVL (mm)	Mass (g)	SVL (mm)	Mass (g)	
Blister (Figure 18)	41.0	5.9	42.7	5.8	- 0.1
Damaged eye (Figure 20)	38.0	5.6	39.9	5.0	- 0.6
Sore on side (Figure 16)	38.0	6.6	40.5	6.0	- 0.6
Red throat (Figure 17)	38.0	5.3	41.1	5.3	No change
Inverted eye (Figure 19)	39.0	5.5	40.7	5.4	- 0.1

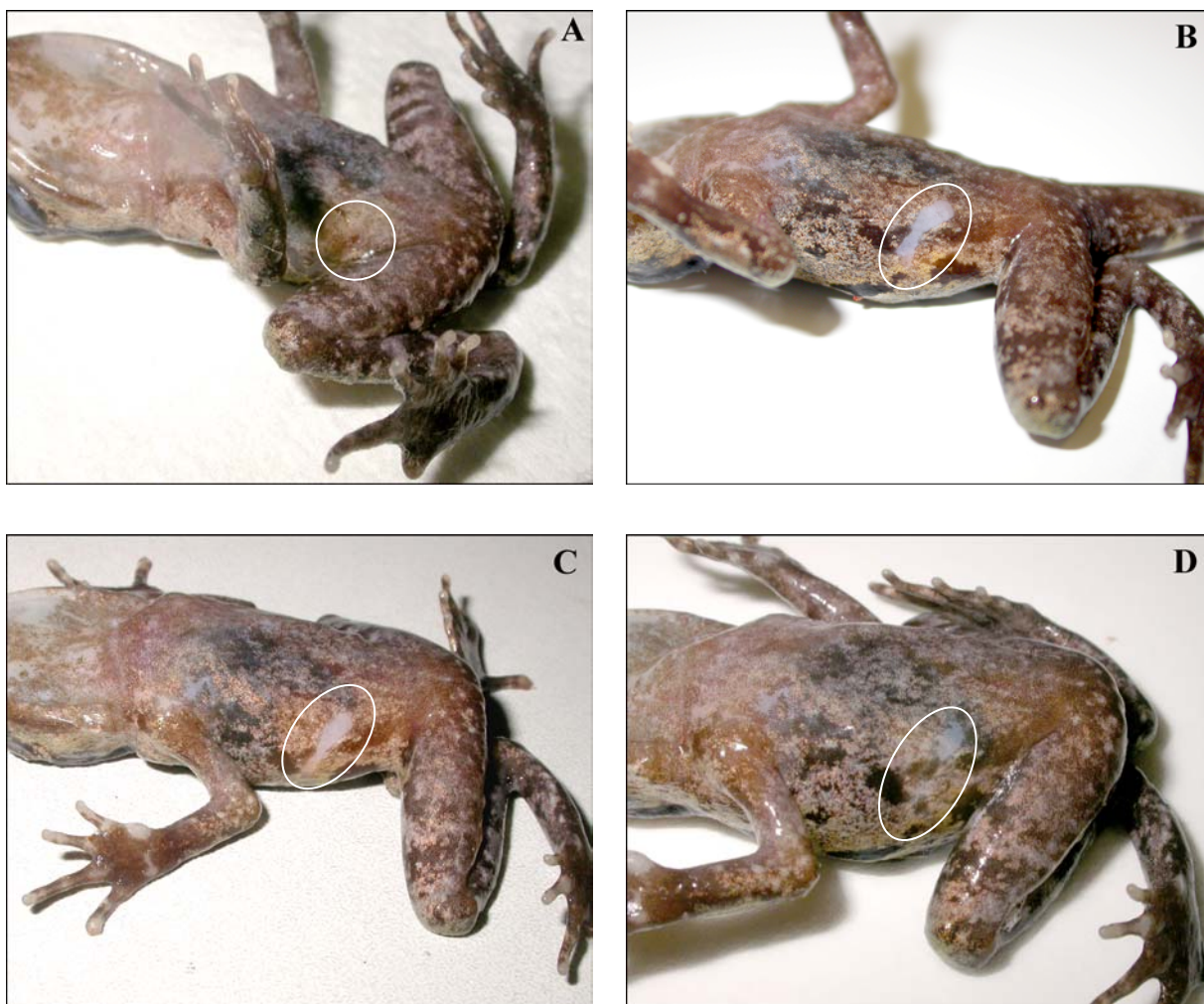


Figure 16 Ventrolateral view of *Leiopelma hamiltoni* photographed at various times over an eight month period, showing lesion development and subsequent denuded area.

A) Specimen on Maud Island, 29 August 2003 – Red inflammation and lesion visible (4 mm^2) (circled); B) Specimen at the University of Canterbury facility, 10 October 2003 –Denuded area clearly visible (11 mm^2) (circled); C) University of Canterbury facility, 7 November 2003 –Denuded area still apparent (28 mm^2) (circled); D) University of Canterbury facility, 20 March 2004 –Partially healed lesion (3 mm^2 & 1 mm^2) (circled)

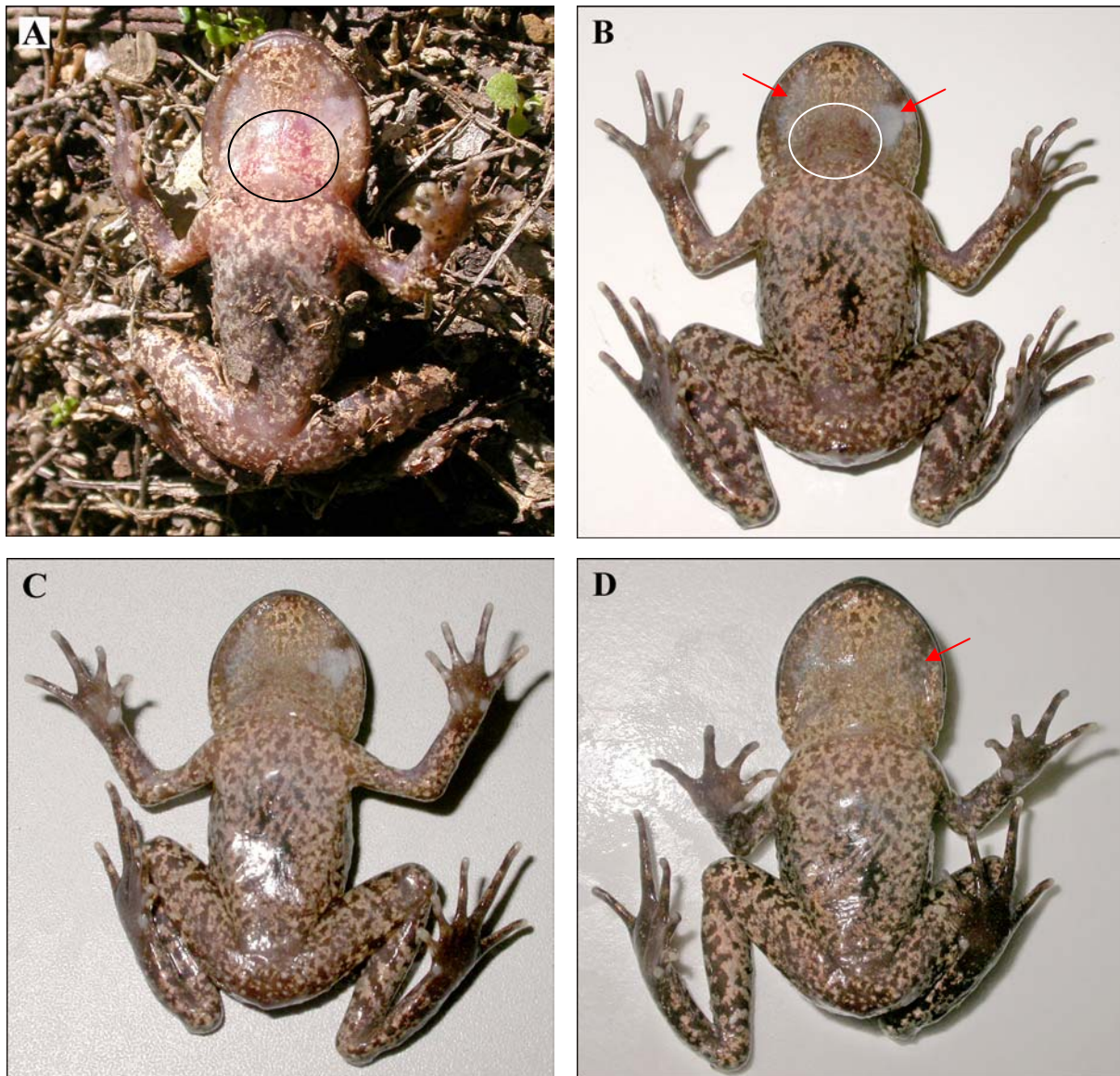


Figure 17 Ventral view of *Leiolopelma hamiltoni*, showing varying levels of intensity of the red throat inflammation and denuded areas at different times during an eight month period. A) Specimen on Maud Island, 29 August 2003 - Red throat distinct (27 mm²) (circled), & denuded (7 mm² & 17mm²); B) Specimen at the University of Canterbury facility, 10 October 2003 – Red throat apparent (9 mm²) (circled) and denuded areas distinct (7 mm² & 17 mm²) (arrowed); C) University of Canterbury facility, 7 November 2003 – Red throat less distinct (4 mm²) but denuded areas still apparent (4 mm² & 14 mm²); D) University of Canterbury facility, 20 March 2004 – No red throat and denuded areas (6 mm² & 6 mm²) (arrowed).

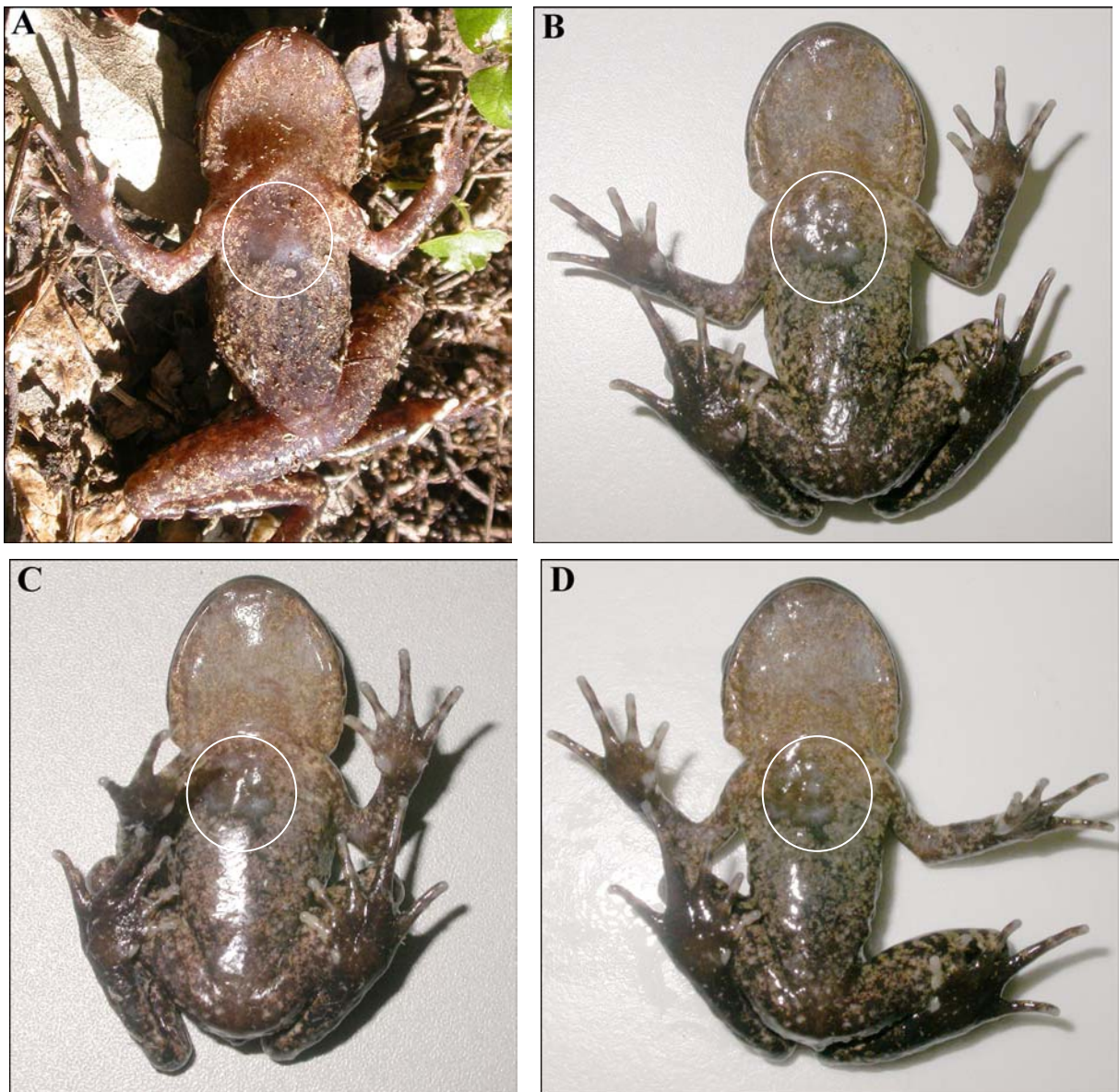


Figure 18 Ventral view of *Leiolopelma hamiltoni*, showing appearance of blister on chest of frog at various times over an eight month period. A) Specimen on Maud Island, 29 August 2003 – Area affected by blisters (54 mm^2) (circled); B) Specimen at the University of Canterbury facility, 10 October 2003 – Blister area more pronounced yet smaller area affected (36 mm^2) (circled); C) University of Canterbury facility, 7 November 2003 – Blistered area size increased (59 mm^2) ; D) University of Canterbury facility, 20 March 2004 – Blistered area reduced (32 mm^2).

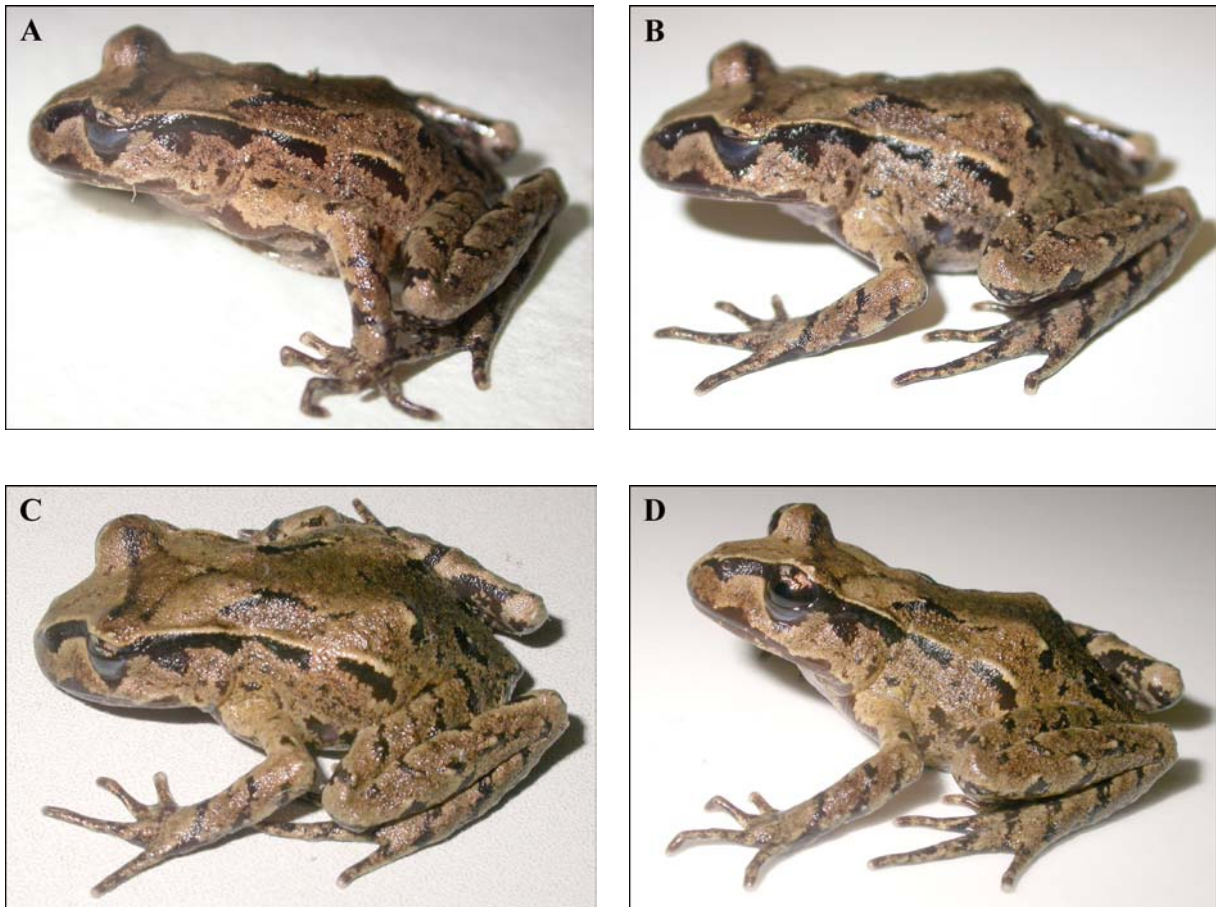


Figure 19 Ventrolateral view of a *Leiolopisma hamiltoni*, showing appearance of left eye at different times during an eight month period A) Specimen on Maude Island, 29 August 2003 – Sunken eye; B) Specimen at the University of Canterbury facility, 10 October 2003 – No change; C) University of Canterbury facility, 7 November 2003 – No change ; D) University of Canterbury facility, 20 March 2004 – Eye in more healthy state.

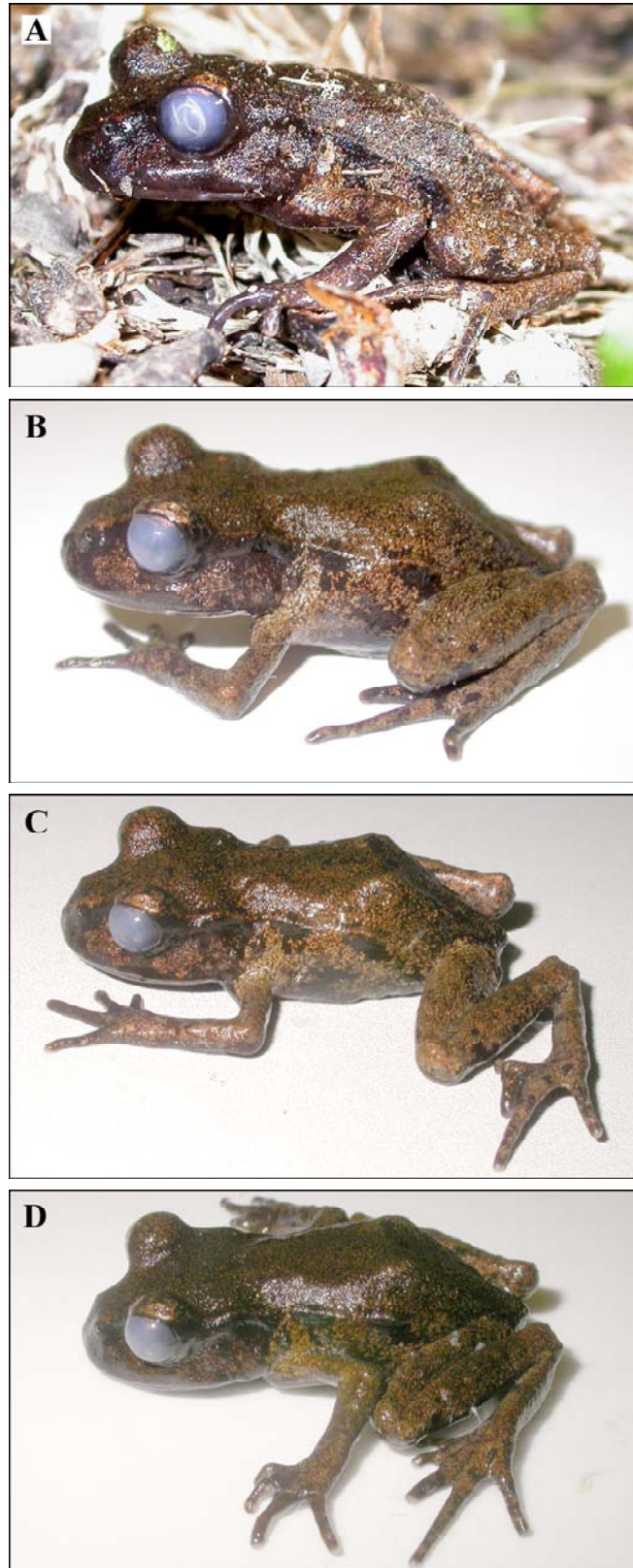


Figure 20 Dorsolateral view of *Leiopelma hamiltoni*, photographed at different times over an eight month period, showing damaged left eye. A) Specimen on Maud Island, 29 August 2003; B) Specimen at the University of Canterbury facility, 10 October 2003 –Cloudy eye; C) University of Canterbury facility, 7 November 2003 - Similar condition; D) University of Canterbury facility, 20 March 2004 – No change.

4.4 Discussion

With the current decline of many amphibian populations (Richards *et al.*, 1993; Laurance *et al.*, 1996; Hero & Gillespie, 1997; Waldman & Tocher, 1998; Daszak *et al.*, 1999; Houlahan *et al.*, 2000; Kiesecker *et al.*, 2001; Davidson *et al.*, 2002; Burrowes *et al.*, 2004) it is important to find ways to monitor the status of amphibian populations. In the past, mark-and-recapture studies have been the commonly used method. However, these only give an approximation of the numbers in a population. The health of the population can be based on how stable the population's numbers are, because steady or increasing numbers imply that mortality and emigration rates are in line with birth and immigration rates. However, basing monitoring techniques solely on numbers does not give any clues as to any underlying status of the population. In the past, populations with apparently large numbers of healthy individuals suffered massive die-offs over short periods of time (Crump *et al.*, 1992; Richards *et al.*, 1993; Lips, 1998; Muths *et al.*, 2003). The use of a photographic record of the population may have given clues to potential problems with the population. The development of a photographic database for captive populations is also important, because it can potentially prevent or reduce the spread of pathogens that may have remained dominant with the host beyond a quarantine period.

Monitoring the health of captive populations also can aid in the management of their wild counterparts, as most captive facilities are very controlled and conditions maintained to remain at the optimal state. With records of both captive and wild populations, comparisons can be made. For example, if something unusual is observed in the wild population as well as the captive population, then it is possible that it is a natural phenomenon. If only one of the populations is showing the symptoms, then it may be an early warning that there is potentially something wrong. The use of a photographic record of bottle nosed dolphins allowed Thompson and

Hammond (1992) to confirm that the strange lesions observed within one population of the dolphins was potentially from a disease. This was done by comparing the photographs of the wild population and those of a captive population. Once it was confirmed that both populations were showing similar symptoms, veterinary records from the captive population were used to confirm that the lesions were a result of a disease.

The results from Figure 12 showed the population of *Leiopelma hamiltoni* on Maud Island had these lesions for many years, since at least 1976 when Don Newman photographed the population to develop a technique for identifying the individuals currently on Stephen's Island (Newman, 1982). However, over the years the number of frogs showing these lesions has increased. These might represent a random chance finding but chi-square analysis suggests otherwise. Alternatively, Figure 14 showed that the lesions are more commonly found on the larger frogs, and as Newman was obtaining repeat samples of the same frogs, the increase in number of individuals with lesions might reflect the frogs increasing in age and size. It is difficult to find the smaller frogs as they are easier to overlook, as they blend into the substrate easily. Therefore the reason that it is more common to find lesions on larger frogs is not related to the actual size of the frog, but rather to the fact that this size class is over-represented in the samples. However, results from the chi-square test comparing the size of the frogs with the presence of lesions indicated that there is a link between them (Table 12). This suggests that if the lesions are caused by a pathogen, the larger frogs are either exposed to the pathogen to a greater extent, or that their immune system is not as strong or effective as that of the smaller frogs.

Although there is variation between years (Figure 13), we cannot be certain that either the number of lesions or the sizes of the lesions increased over time. Some variation may have arisen from random sampling but chi-square analysis demonstrated that the overall trends were non-random (Table 11). This observation does not necessarily point to disease as the cause, as there

may be some other, as yet unknown factor that affects and controls the outbreak of these lesions. The information shown here does indicate that future monitoring of this population and the formation of the lesions is warranted as a precautionary measure.

Comparisons of Figure 12 and Figure 13 show large increases in the number of frogs with lesion symptoms for 2003 and 2004 once the ventral region of the frogs is taken into account. This suggests that the data shown for the years monitored prior to 2003 have not shown the full extent of the lesions, as ventral views of the animals were not available meaning it was not possible to analysis the extent of the lesion on the ventral surface (Figure 12 & Figure 13). The reason for this may be that the causes of the lesions are due to opportunistic pathogens and that the ventral region of the frogs are more susceptible to injuries considering that they use rock pile as retreat sites. Alternatively the lesions observed on the ventral region of the frogs might not be the same as the lesions observed around the face and legs of the frogs, and the ventral lesions might simply be due to the wear and tear that the frogs' skin is subjected to on a daily basis.

The monitoring of the sick captive frogs suggests that the frogs appear to be able to cure themselves of the denuded patches and the red colouration on the throat region. The causes behind one of the frogs having an eye sunk into its socket is still unknown; however, as the eye appears to have returned to normal this does not seem to be a potential problem for the population. The cause of the damage to the eye of the frog which appears to be blind in one eye is also unknown and may warrant further research.

The improvement of the frogs that showed a change in their condition may have been a result of removing these animals from the environment and therefore, preventing re-infection or multiple infection events occurring, resulting in a level beyond the capabilities of the frogs' immune systems. It may also be possible that the captive environment is not suitable for the

pathogen to survive in. Animals may be subjected to factors in the wild that cause immunosuppression that prevent them from coping with the cost of pathogens, but this is ameliorated in a sterile laboratory environment. It is possible that these symptoms are caused by an opportunist pathogen that is commonly found on the frog's skin or in the frogs' habitat. Possibly, the causative agent of the lesions may not be fatal but that the lesions represent a secondary effect from either another pathogen or environmental effects, or come about due to increased competition as an area becomes overpopulated.

The observations for frogs with the blisters and the sore on its side (Figure 18 and Figure 16) showed that the affected areas become larger within the first period of their arrival in captivity. This suggests that stress may play an important role in the frogs' ability to fight off these infections. A frog may be exposed to increased stress levels in many ways, such as by increased exposure to dry conditions, decrease in food resources or increase in competition for these resources, or finally other diseases, which may weaken the immune system. However, none of these factors appear likely to explain progression of symptoms in the captive facility.

The lesions on the frogs' skin may not be the result of a disease, but rather indicate some diet deficiency. This deficiency may be either purely a lack of food or alternatively a certain component within their diet. High competition between individuals may result in the weaker animals missing optimal prey species. The fact that the lesions decreased in size after being in captivity might indicate that diet, either quality or quantity, may affect the formation of the lesions. However, observations of the various frogs with lesions showed that while some frogs had a small girth, other frogs had large girths, suggesting that the lesions are not the result of a lack of food. It may be possible that the lesions are a consequence of the breeding season; either due to the high energy required for the females to produce the eggs, or that the males' feeding efficiency is compromised while protecting the eggs. If the other population of *L. hamiltoni*

show similar lesions, then comparisons of both the prey type and quantity available for each different population may indicate if there is a link between the diet and the lesions.

The development of a photographic record is an effective way of ensuring that all observations are available to everyone who is working with the various populations. There is always the possibility that different observers are going to interpret different things as important or not, and the development of a photographic record will allow different people to compare their observations with those of others. These observations may be from different populations or from different years. However it is important to establish clear guidelines of what information needs to be obtained from each photograph as the number of frogs showing lesion symptoms increased during 2003 and 2004 when the ventral view was included. Through developing a standardised sampling and recording methodology it is possible that a database containing all individual frogs recorded will be able to be established.

The ability to monitor the populations' health status will aid in the management of these species. The next important step would be to photograph the Motuara Island population, which was started from 300 Maud Island individuals, the population of Stephen's Island and the recently translocated individuals on the Chetwood Island, to see if any of the frogs within these populations have the same lesions. This may answer the question as to whether this is a natural phenomenon and all the frogs within that species are susceptible, or whether it is restricted to the Maud Island population.

If it is restricted to Maud Island, this may indicate that there is a pathogen on the island which may impact future translocations. However, if pathogens are on the island, it would be highly probable to expect the Motuara Island population also to be infected and showing the symptoms, as they were only translocated in 1997 and the past photographs indicated that these symptoms

have been present in the population since at least 1976. If the Motuara Island population does not show these systems it may suggest that the lesions are a side affect of over-crowding. A possible way to confirm this would be to observe the population in Boat Bay, Maud Island. This population were translocated from the main forest reserve prior to 1997 (Bell *et al.* 2004). The only variation between this population and the main population is the number within an area.

The development of a photographic record for monitoring the health of *L. hamiltoni* is an effective method of ensuring that all populations of this species remains in optimal health to help increase their long-term survivorship and that of other *Leiopelma* species.

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Chapter Five

Discussion

5.1 Discussion

In recent years there have been mass declines of many amphibian populations on a global scale (Richards *et al.*, 1993; Laurance, 1996; Hero & Gillespie, 1997; Waldman & Tocher, 1998; Daszak *et al.*, 1999; Houlahan *et al.*, 2000; Davidson *et al.*, 2002; Burrowes *et al.*, 2004), and unfortunately some species have not been seen for many years and are now possibly extinct (Towns, 1994; Lips *et al.*, 2003; Santiago *et al.*, 2003; Burrowes *et al.*, 2004). Over the years there have been many hypotheses proposed to explain why amphibian populations are declining.

Disease outbreaks are a widely accepted hypothesis explaining the decline of many populations (Carey, 1993; Crawshaw, 1997; Daszak *et al.* 1999; Bosch *et al.* 2001; Lips *et al.* 2003; Muths *et al.* 2003). Chytrid fungus is a commonly known disease which is found on sick or dying frogs, and as a result has been reported many times to be the cause of various population declines (Carey, 1993; Crawshaw, 1997; Daszak *et al.* 1999; Bosch *et al.* 2001; Waldman *et al.* 2001). However, there is the serious danger of linking population declines to diseases when there is the possibility that they are the secondary consequence of a larger problem.

An alternative hypothesis proposed for amphibian population declines is habitat destruction (Drost & Fellers, 1996; Johnston & Frid, 2002). Although there is evidence that habitat loss through forest fragmentation has not affected some populations (Chazal & Niewiarowski, 1998; Silva *et al.*, 2003), this does not discount the hypothesis that habitat loss is the cause for the declines of some amphibian populations.. It is possible that some of the declines that have been attributed to disease may in fact be a consequence of a decrease in home range size due to a loss of suitable habitat. Johnston and Frid (2002) showed that some amphibians are

indeed affected by loss of habitat. Pacific giant salamanders, *Dicamptodon tenebrosus*, have been significantly affected by clear-cut logging. Salamanders in the clear-cut areas spent more time in subterranean retreat sites and had smaller home ranges than the individuals within the forested area and the riparian buffer strips. Also, the salamanders in the clear-cut area were more dependent on precipitation than the populations in the forested areas and the riparian buffer strips. This may be the result of human impacts or it may indicate that the individuals found within the clear-cut areas cannot obtain and defend sustainable home ranges, due to a reduced fitness or resource availability (e.g. food).

Research on home range size and requirements is needed for many amphibian populations, as some of the current declines may be a result of either increased competition for home ranges or decreases in the size of home ranges, because of increased population density due to a lack of suitable habitat. This may have resulted in the reduction of the availability of resources needed for survival or attracting a mate (Kleeberger, 1985), and potentially may have affected the long term survival of the population.

Current population estimates based on mark-and-recapture studies may not be sufficient to detect changes in population dynamics and habitat usage. For this reason, it is important that population studies are conducted on a finer scale, and that monitoring of home ranges is used as a way of determining potential changes in population dynamics.

It is difficult to study the home range of many amphibian species because of the habitat in which the amphibian lives or their life-history traits. However, *Leiopelma hamiltoni* seems to be ideally suited for home range studies because it is an entirely terrestrial frog (Bell, 1982; Newman, 1982) and its behavioural traits, of freezing when threatened allows for photographs to be easily taken, and the habitat in which it is found. *L. hamiltoni* is restricted to three small

offshore islands, with the majority of the population found on Maud Island within a 16 hectare forested area.

By establishing a grid within the forest on Maud Island it was possible to track the movement of several *L. hamiltoni* individuals. All of the locations where each of the individuals being tracked within the grid were found were translated into Cartesian co-ordinates and entered into the computer program, Homerange (Huber & Bradbury, 1999). The program plotted the home ranges for each of the individuals, and based on the minimum complex polygon method (MCP) it showed that the home range of *L. hamiltoni* may vary from 0.5 m² to 25 m². The MCP home range size is based on the smallest polygon shape that can be produced by using all the outer most data points as a guideline for the shape. With the exception of frog H, all the remaining frogs showed varying degrees of overlap of their home ranges. The home range of frog H overlapped slightly with the bottom of frog I. It is possible that levels of relatedness affected the degree of home range overlap. Waldman & Bishop (2003) found that frogs showed a greater preference for the substrate that they had marked themselves, compared to substrate marked by a frog collected further away. The frogs showed no preference when the substrate had been marked by a neighbour, which may mean that if closely related individuals are sharing home ranges, then they may be familiar with the odour of their relatives. However, as there currently is no information to suggest that *L. hamiltoni* is territorial, it is possible that a high population density on Maud Island has resulted in substantial overlapping of home ranges, and that due to this overlap the frogs have become familiar with the odours of the neighbouring frogs, as they come in contact with these odours regularly. In contrast, odour used from another frog beyond its own home range would be unfamiliar to the frog.

With the vast majority of the entire population of *L. hamiltoni* being on one island, it is important that any research conducted on this population is done in such a manor that it does not

increase any potential detrimental risks. One of the most commonly used methods for identifying *L. hamiltoni* is through toe clipping. However, as toe clips can be a potential entry point for opportunistic pathogens (Doody, 1995; Davis & Ovaska, 2001) and impact of the return rate depending on the number of toes clipped (McCarthy & Parris, 2004), it is important that alternative methods are established, because if a pathogen became established within the Maud Island population, the results may be dramatic declines from which recovery is not possible.

The use of natural markings within amphibian research is starting to be used more commonly, although is still limited to a few species (Newman, 1982; Doody, 1995; Loafman, 1991; Donnelly *et al.*, 1994; Meyer & Grosse, 1997; Bradfield, 2004). Doody (1995) used the number and appearance of the dorsum bars on *Ambystoma opacum*, individuals. Once the number of bars was known, individuals could be identified without comparisons to photographs being needed for confirmation.

One of the major problems with natural markings as a method of individual identification is the long-term reliability of the markings. It is important that the markings used do not change over time or as the animal grows. The markings also need to be clearly distinguishable to prevent confusion, yet easy enough to describe and explain to multiple users. Meyer & Grosse (1997) showed that the spot patterning on the ventral surface of *Bufo calamita* could be used after the first hibernation, as the patterning became stable after this time. The patterning used by Meyer & Grosse (1997) remained stable for the duration of the five years the individuals were being monitored. With the knowledge of the timeframe over which the markings remain stable, it means that patterning changes can regularly updated and the changes should not impact identifying individuals based on their patterning.

Once the stability of various landmarks is established, it is possible to begin developing a technique by which the individual can be identified. Through comparison of historical records it

was shown that there are some landmarks on *L. hamiltoni* that do not change with either age or growth of the individual (chapter 2). This means that these markings can be used for extended periods. There have been several previous attempts to develop a technique to individually identify *Leiopelma* species. Newman (1982) developed a technique based on the landmark patterning on the lips, to enable the small population of *L. hamiltoni* on Stephen's Island to be individually identified, and more recently, Bradfield (2004) has developed a technique to identify *L. archeyi*. However, the long-term reliability of Bradfield's (2004) technique at this stage is unknown, as information with regards to the stability of the landmarks has yet to be determined.

The landmarks that were used for individually identifying *L. hamiltoni* were the darker pigmented area on the surface of the skin, on the facial region and the lateral lines on both sides of the animal. Each of the landmarks was used for a different stage of the classification process. Due to the differing characteristics of each of these landmarks they were able to be encoded three different ways. Through testing of the classification process based on the description of the landmarks and a set of photographs, the frogs were correctly placed into the same category 76 % of the time.

However, once the frogs were already classified into the different sub-groups, the live frogs were used to test the success rate of the frog being re-identified, by comparing the live frog to all photographs in the same sub-group. It is very important that the frogs are able to be identified accurately once they have already been placed into sub-groups as this represents recaptures. If the technique was to be used to sample the same area over an extended period of time, then there is the need to be able to clearly say if a frog has been sampled before or if it is possible be a new recruit to the area. The mean accuracy for this process was 93 % correct. These results show that it is possible to accurately identify *L. hamiltoni* based on natural landmarks.

During the initial stages of developing a technique for individual identification, unusual denuded (depigment) patches were observed in the individuals recently arrived at the captive facility at University of Canterbury. Initially it was thought to be an artefact of being removed from the wild (B Waldman, personal communication). However, further observations were made of photographs of the wild population, and it was shown that these patches were present. This served as impetus for us to develop a photographic record to monitor the health of individuals.

The five *L. hamiltoni* that were removed from the wild to enable skin changes, possibly attributable to disease, to be monitored were photographed regularly. The original photograph was taken while the frogs were still on the island. The frogs were re-photographed within the first month of arriving at the captive facility at the University of Canterbury. Over the next seven months the frogs were photographed twice more. Through the use of ImageJ the size of the denuded patches on each of the frogs were measured and the comparisons of the changes were made over the eight month period. Through developing a photographic record of these frogs it was possible to use photographs to monitor the health of *L. hamiltoni*. Although, the frog with the damaged eye showed no improvement with regards to the eye, all other frogs' conditions improved over time. The frog that had the sore on its side showed the most improvement. The sore started as a small wound on the side of its body, by the time the second photograph was taken the sore had changed to a large denuded patch. Upon close examination, the change in the thickness of the skin became apparent. The skin had become transparent. During the next two recordings the wound showed signs of improvement, with the final photograph showing the affected area had return to normal pigmentation colouring.

While the monitoring of *L. hamiltoni* shows that diseases and injuries can be monitored through the development of a photographic record, Wright & Zamudio (2002) used colour pattern asymmetry to test the effect of habitat disturbance on *Ambystoma maculatum* (spotted

salamander). Samples were collected from the historical specimens held at the Cornell University Museum of Vertebrates, with that of live specimens from an undisturbed site of a protected wetlands, and an area that had been disturbed by the development of a golf course. Recordings of spot asymmetry were taken from both live animals and photographs. By comparing the spot asymmetry of all the various populations, the researchers showed that the salamanders in the disturbed habitat may have experienced higher stress and this was reflected in the degree of spot asymmetry. Wright & Zamudio (2002) concluded that even though there were still salamander populations in the disturbed habitat, it did not guarantee their long-term persistence. This shows that the development of a photographic record can also be used to monitor the impact habitat disturbance is having on the affected populations, which is one hypothesis proposed to explain current declines of amphibian populations (Drost & Fellers, 1996; Johnston & Frid, 2002).

However, regardless of the reason for developing a photograph to monitor the health of a population, a database will be required to enable information to be easily accessed, and also for the maintenance of records to be up dated regularly. However, it is possible to use the same database that is used for individual identification purposes. The advantage of using the same database for the development of an early warning system for disease as that used for individual identification is that it reduces both the frequency and duration at which the frogs are handled and photographed, therefore reducing the stress experienced by the frogs (Coddington & Cree, 1995). It is also possible that if different people are working with different population, that they do not need to have direct contact with the other population, it reduces the risk of diseases being spread between populations, yet there are still data available from the other populations.

However, if a database were to be established there would need to be guidelines as to the information that would need to be attached to each photograph. For example, date, location, SVL, and which version of photograph it is (especially important for captive population where regular

photographing is possible), and a code so it can be quickly identified. For each of the different individuals within the database, there would need to be the exact same views available (e.g. face, rump, ventral, dorsal, both lateral views, and if it was showing signs of illness close up view of the symptoms). The ideal situation would be to have the entire photographic database available nationally, allowing everyone access to the information.

5.2 References

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